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EFFECT OF RATE OF SEEDING UPON COMPARISON OF VARIETIES OF OATS¹

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Abstract

Three varieties of oats: Abundance, Banner and Daubeney, were sown at various rates in 1924-25-26 at four places differing widely in soil and climate, *viz.*, Charlottetown, P.E.I., Macdonald College, Que., Scott, Sask., and Edmonton, Alberta. Daubeney has smaller seed and tillers more freely than the other two varieties.

The method of field experimentation adopted is considered in the light of the principles of "randomisation" and "local control" developed at Rothamsted by R. A. Fisher. The plots sown at each rate were adequately replicated but their systematic arrangement precluded a valid estimate of the errors in yield comparisons. In particular, the arrangement of the plots sown at different rates was shown to coincide to some extent with variations in soil fertility.

In general the differences in yield between plots of the same variety sown at different rates were small. Daubeney showed the greatest variations; the expected stabilizing effect of its tendency to tiller did not appear.

The combined results of the three years indicate that the optimum rate of seeding may not be the same for different varieties at the same station, and certainly is not the same for the same variety at different stations. Near the optimum the effect of variations in seed rate upon yield were slight and, at three out of the four stations, all three varieties might be sown at a specified uniform rate without significantly increasing the ordinary experimental error.

Large fluctuations were found in the percentage stand of plants recorded, due in part to unavoidable errors in counting, but still indicating significant differences between the stations. At three stations the optimum stand of Daubeney may have been higher than that of the other two varieties, though at only one station is the difference significant.

1. Introductory

The important place occupied in Canadian agricultural science by that type of field experimentation known as "variety trials" is well known. Not only are such trials essential in determining the suitability, to any particular set of environmental conditions, of the very many strains and varieties of all important crops which now exist, they are also the only means at our

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² A statistical study of experimental data accumulated by the Associate Committee on Accurate Plot Work during the seasons 1924-25-26. The membership of the Committee and brief descriptions of the co-operative work accomplished are given in the Annual Reports of the President of the National Research Council of Canada for the years ending March 31, 1925-26-27.

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disposal for discovering the true merits of those productions of the geneticist which have played so prominent a part in the extension of agriculture to regions hitherto held unsuitable, or, less spectacular but equally important, have materially increased the productivity of areas already under cultivation. There can be few agricultural experiment stations in the country not at the present time actively engaged in field trials of this nature.

Notwithstanding the extent to which such experimentation has been and is being carried on, some uncertainty has existed as to the exact procedure by which the desired result, namely, the unbiased comparison of the productive capacity of different strains of the same crop, might best be attained. As a result, there has been considerable divergence of practice (2). It was in view of the obvious and important advantages accruing from any increase in uniformity of procedure and accuracy that the present investigation, which represents an attempt to resolve some at least of the points at issue, was undertaken.

The difficulties here investigated centre around the fact that the varieties to be compared usually differ in many other characters besides inherent yielding capacity. One of the most obvious of these is size of seed.

Size of seed, or weight per 1000 kernels, which is the quantitative measure usually adopted, is known to vary considerably from variety to variety. It also varies from sample to sample of the same variety, depending upon the soil and climatic conditions under which the seed was produced. In consequence, the Swedish experimental stations have long adopted the principle, which also has its adherents in Canada (13), that in comparative tests equal numbers of germinable seeds should be applied to equal areas of land.

This principle has never gained any wide acceptance on this continent, where the common practice is to sow equal *weights*, rather than equal numbers of seeds. Experience here also indicates that the Swedish procedure does not sufficiently take into account two facts: that a really fair comparison of productive capacity is possible only when each variety is seeded at its *optimum* rate, and that this optimum rate may be influenced by size of seed as well as by other factors.

For example, a large number of experiments have been performed to test the yielding capacity of large and small seed of the same variety. Summarizing all such data then available dealing with small grain crops Kiesselbach (12) found that, when both were planted in equal numbers at a rate optimum for the large seed, the small seed yielded 11% less grain per acre than the large. When equal weights were planted, however, again at a rate optimum for the large seed, the small seed yielded only 3% less. It would thus appear that, quite apart from the varietal factor, small seeds should be planted in greater numbers than large if they are not to be placed at an unfair disadvantage.

The fact that different varieties may have considerably different optimum seed rates has been observed in the case of corn, wheat and oats. Mooers (14), speaking of corn, says, "On the same land different varieties of similar length of season and habit of growth may differ appreciably in the rate of planting which gives best results". Osborn (15), also working with corn, noted certain

varietal differences in response to increased seed rate. He found that the optimum rate of seeding was influenced by the fertility of the soil, and gave it as his opinion that no specified rate of planting of the same variety could be depended on to produce maximum yields in successive seasons even on the same character of land, owing to changed climatic conditions. Grantham (8) found considerable differences in the tillering capacity of wheat varieties. When varieties differing in this respect were sown at three different rates (equal numbers of seeds of the eight varieties being sown in each case), marked varietal differences were found in response to increase in rate of seeding. Stanton *et al.* (16) have carried out a rate of seeding experiment with fall-sown oats at Athens, Ga. Two varieties, Red Rustproof and Fulghum, were used. The optimum rate for Red Rustproof was found to be six pecks, whereas the yield of Fulghum increased progressively with the seed rate up to a rate of 10 pecks, the highest used, thus indicating that the optimum *weight* of seed may be no more constant than the optimum number.

We therefore perceive that the optimum stand of any crop under a given set of conditions may be influenced by a number of factors—morphological, physiological, and environmental—which may be expected to interact in a complicated and often obscure manner. In consequence it would appear that an investigation into the optimum stand of crops under different conditions of soil and climate might do much to put variety testing on a sounder scientific basis.

In 1923 the National Research Council of Canada appointed an Associate Committee on Accurate Plot Work, with representatives of the Federal Department of Agriculture and of various agricultural colleges. Certain members of this committee drew up a scheme of investigation into the effects of rates of seeding upon the comparison of varieties of oats, the experimental work being carried out during the seasons 1924, 1925 and 1926. The Dominion Cerealist, as Secretary of the Committee, collected and filed each year the experimental data thus obtained; these form the basis of the present report.

2. Experimental Procedure

In addition to its importance in Canadian agriculture, oats had certain obvious advantages for the purposes of this experiment. Those variations in seed size, the effect of which it was desired to study, are here pronounced, and in addition there are often marked differences between varieties in respect of tendency to tiller.

To achieve the objects of the investigation it appeared that experimentation should be conducted at a number of places in different parts of the country. This should enable the effect of variations in soil and climatic conditions to be assessed and conclusions of some generality to be obtained. Furthermore it was recognized that too much reliance could not be placed on the results of a single season's work. It was therefore decided that the experiment should be carried on for a period of three years.

Three varieties of oats were selected for experimentation: Abundance, a variety supposedly characterized by fairly large seed and low tendency to

tiller; Banner, a variety medium in size of seed and tendency to tiller; and Daubeney, a small-seeded variety with pronounced tillering propensities.

The general plan supplied to all co-operating stations showed the plots laid out in three long ranges, one range being devoted to each of the experimental varieties. In practice, each station adapted the scheme to its local field arrangement. At the University of Alberta, for example, the experimental field is laid out in blocks about eight rods wide. The planting of the oat plots was begun at one corner of such a block and carried back and forth alternately across this distance until one variety was completed, when the next variety was immediately begun. The whole experiment was thus contained within an area about 8 by 11 rods in size.

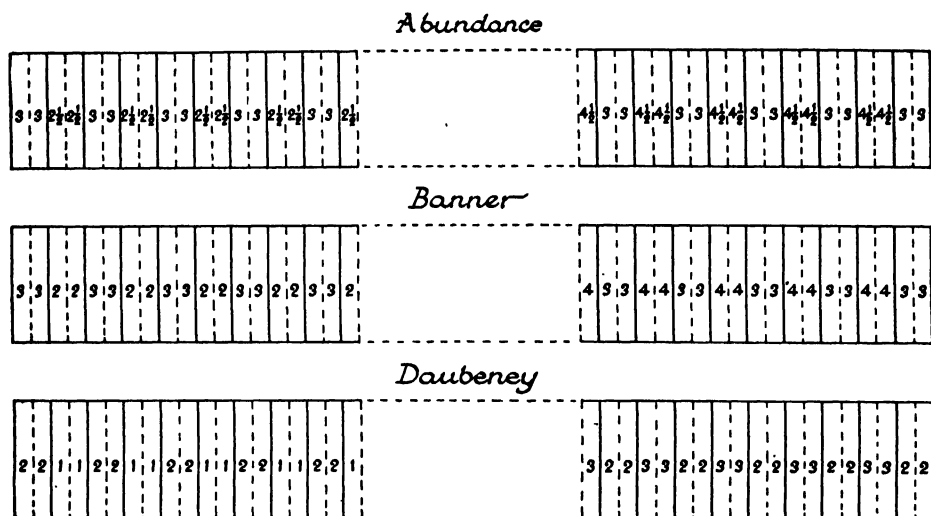


FIG. 1. Scheme of arrangement of plots in the field.

Each plot consisted of nine drills $18\frac{1}{2}$ ft. long. At harvest time one foot was removed from each end of the plot to eliminate "border effect", the plot actually harvested being thus exactly one rod in length. The drills were spaced 7 in. apart, except at Macdonald College, where one link was the spacing adopted. In addition to the removal of one foot from each end of the plot, the two outside rows were also discarded at harvest time. Of the remaining seven rows, the central one was dug up in its entirety and the number of plants and tillers contained therein determined; no other use was made of this row. The remaining three rows on either side of this central row were harvested separately in the form of two three-row plots. Each rate of seeding (of which there were four in the case of each variety) occupied eight such nine-row plots, or sixteen of the three-row plots.

The allocation of the plots to the various rates of seeding proceeded in a systematic manner, the eight plots bearing the lowest rate of seeding being grouped together at one end of the block, then the eight plots of the next highest rate, and so on. "Check plots", sown at a rate constant within each

variety, were however alternated with the rate plots, so that each three-row "rate plot" was immediately adjacent to a "check plot" of similar size; it being believed that by utilizing the *differences* in yield between such adjacent plots, rather than the actual yields themselves, the precision of the experiment would be enhanced. The whole experiment was in fact laid out with the object of obtaining a series of differences in yield between adjacent "check" and "rate" plots, the significance of which could be tested by the well-known method first propounded by "Student".

The total number of large, or nine-row, plots in each "range" amounted to 65, allowing for a check plot at each end. The general plan of arrangement is illustrated diagrammatically in Fig. 1. The complete series of rates, in the order in which they occurred, was as follows:

Range 1. Banner				Range 2. Daubeney				Range 3. Abundance			
Plot	Rate, bu. per acre	Plot	Rate, bu. per acre	Plot	Rate, bu. per acre	Plot	Rate, bu. per acre	Plot	Rate, bu. per acre	Plot	Rate, bu. per acre
1	3.0	34	3.5	65	2.0	98	2.5	129	3.0	162	4.0
2	2.0	35	3.0	66	1.0	99	2.0	130	2.5	163	3.0
3	3.0	36	3.5	67	2.0	100	2.5	131	3.0	164	4.0
4	2.0	37	3.0	68	1.0	101	2.0	132	2.5	165	3.0
5	3.0	38	3.5	69	2.0	102	2.5	133	3.0	166	4.0
6	2.0	39	3.0	70	1.0	103	2.0	134	2.5	167	3.0
7	3.0	40	3.5	71	2.0	104	2.5	135	3.0	168	4.0
8	2.0	41	3.0	72	1.0	105	2.0	136	2.5	169	3.0
9	3.0	42	3.5	73	2.0	106	2.5	137	3.0	170	4.0
10	2.0	43	3.0	74	1.0	107	2.0	138	2.5	171	3.0
11	3.0	44	3.5	75	2.0	108	2.5	139	3.0	172	4.0
12	2.0	45	3.0	76	1.0	109	2.0	140	2.5	173	3.0
13	3.0	46	3.5	77	2.0	110	2.5	141	3.0	174	4.0
14	2.0	47	3.0	78	1.0	111	2.0	142	2.5	175	3.0
15	3.0	48	3.5	79	2.0	112	2.5	143	3.0	176	4.0
16	2.0	49	3.0	80	1.0	113	2.0	144	2.5	177	3.0
17	3.0	50	4.0	81	2.0	114	3.0	145	3.0	178	4.5
18	2.5	51	3.0	82	1.5	115	2.0	146	3.5	179	3.0
19	3.0	52	4.0	83	2.0	116	3.0	147	3.0	180	4.5
20	2.5	53	3.0	84	1.5	117	2.0	148	3.5	181	3.0
21	3.0	54	4.0	85	2.0	118	3.0	149	3.0	182	4.5
22	2.5	55	3.0	86	1.5	119	2.0	150	3.5	183	3.0
23	3.0	56	4.0	87	2.0	120	3.0	151	3.0	184	4.5
24	2.5	57	3.0	88	1.5	121	2.0	152	3.5	185	3.0
25	3.0	58	4.0	89	2.0	122	3.0	153	3.0	186	4.5
26	2.5	59	3.0	90	1.5	123	2.0	154	3.5	187	3.0
27	3.0	60	4.0	91	2.0	124	3.0	155	3.0	188	4.5
28	2.5	61	3.0	92	1.5	125	2.0	156	3.5	189	3.0
29	3.0	62	4.0	93	2.0	126	3.0	157	3.0	190	4.5
30	2.5	63	3.0	94	1.5	127	2.0	158	3.5	191	3.0
31	3.0	64	4.0	95	2.0	128	3.0	159	3.0	192	4.5
32	2.5	64a	3.0	96	1.5	128a	2.0	160	3.5	193	3.0
33	3.0			97	2.0			161	3.0		

All plots were sown by hand, a definite *weight* of seed being placed in each drill. All seed used was of common origin, being supplied by the Dominion Cerealist, Central Experimental Farm, Ottawa. Information regarding the weight per 1000 kernels and percentage germination was also supplied for the

seed of each variety each year. These values, however, were checked in most cases by the individual stations, at the request of the Dominion Cerealists, and in some cases where the divergence of results was appreciable, the stations used their own figures in calculating the number of germinable seeds sown in each plot.

The experimental work was commenced in 1924. An invitation to participate was issued to a number of experimental stations and agricultural colleges, at the following eight of which the experiment was actually carried out during the first season:

Experimental Station, Charlottetown, P.E.I.

Experimental Station, Cap Rouge, Quebec.

Macdonald College, Quebec.

Central Experimental Farm, Ottawa, Ont.

University of Manitoba, Winnipeg, Man.

Experimental Station, Scott, Sask.

University of Alberta, Edmonton, Alberta.

University of British Columbia, Vancouver, B.C.

Owing to a variety of causes the number of the co-operating stations progressively declined during the two remaining seasons of the experiment. The present report is confined to the analysis of the data secured at four stations, *viz.*: University of Alberta, Scott Experimental Station, Macdonald College, and Charlottetown Experimental Station. Fortunately, these stations are very widely separated and represent fairly well the maximum range of soil and climatic conditions likely to be met with in field experimentation in Canada. At all four stations the field operations would appear to have been carried out with all reasonable care, including, at Scott, the surrounding of the area occupied by the plots with wire netting, to prevent the augmentation of the experimental error by the molestations of gophers and rabbits. It is necessary to note, however, that at Macdonald College the variety Abundance was not grown in 1925, and that at Scott in the same year a departure from the general practice in determining the stand of plants and the number of tillers occurred. This took the form of counting the stand of plants in the centre row of each plot in the spring, as soon after emergence as possible. At harvest time, a further count of the total number of culms was made, the difference between these two counts furnishing the estimate of tillering. Whilst any departure from uniformity of procedure would have been regrettable, this one proved especially so owing to the fact that by no means all the plants which emerged survived to maturity. In consequence, no weight can be attached to any estimate of tillering thus derived. Indeed, in some cases the total number of culms counted in the autumn was less than the number of plants counted in the spring.

3. The Principles of Field Experimentation Developed at Rothamsted by R. A. Fisher

It is of course now very widely recognized amongst all thoughtful persons engaged therein, that field trials, in common with most other forms of experimentation, particularly those of a biological nature, should be at least duplicated

and preferably further replicated. The precise nature of the benefits to be derived from this procedure is not perhaps so generally realized. These are in fact twofold. In the first place, since the mean of several determinations is more reliable than a single determination alone, replication increases the *precision* of our results. Secondly, by an examination of the variability of replicate observations, we are enabled to arrive at an estimate of the *experimental error* to which the results are subject. It is only by considering any observed differences in the light of this error that we may arrive at conclusions of any scientific value. The peculiarity of field experimentation lies in that, whilst the second of these considerations, the estimation of error, is here of particular importance, yet the methods adopted have often not been such as to ensure the validity of any such estimate obtained.

This condition of affairs is due to the fact, suggested by a wealth of observational evidence and verified in all careful uniformity trials (9), that any area of land chosen for experimental work may be assumed to be more or less heterogeneous from the point of view of fertility. Moreover this variation, although often extremely complex, nevertheless usually exhibits a systematic element, in the sense that if the whole area were divided into a large number of sub-areas, the fertility of neighboring sub-areas would tend to be positively correlated. In short we have what "Student" has termed "a sort of regular irregularity". It appears that in most agricultural experiments soil heterogeneity is by far the most potent cause of variation in the yields of similarly treated plots. By the exercise of reasonable care the effect of errors in seeding, harvesting, weighing, etc., may be reduced to quite secondary proportions.

From these considerations it is obvious that all experimental design should have two objects. The first is to minimize the disturbing effect of soil heterogeneity upon the comparisons which it is desired to make. The second is to render possible a valid estimate of the errors to which our comparisons, as performed, are subject.

Now the requirement of a valid estimate of error is that the differences in fertility between parallel plots (*i.e.*, plots treated alike) should be representative of the differences in fertility between plots treated differently. It is clear that we have no right to assume that we have achieved this desideratum if any systematic arrangement of plots is superimposed upon land of which the fertility varies from point to point in a manner which also has in it a systematic element. The two systems may well have features in common, in which case our estimate of error will not be of valid application. The direct way to overcome this difficulty according to Fisher (4, 5, p. 229 *et seq.*), is to arrange the plots wholly at random. If this is done, the error, as estimated from plots treated alike, will be a valid estimate of the error affecting comparisons between plots treated differently. The principle of *randomness* should therefore be regarded as an essential condition which any plot arrangement must fulfil.

It is not of course contended that the estimate of error obtained from any random arrangement necessarily coincides with the true error of the experiment. If it did, it would be more than an estimate. But if we imagine the experiment to be performed, under identical soil and climatic conditions,

using all the equally possible random arrangements, then it would be found that the estimates of error thus obtained were clustered around a central value, this central value being the true error of the experiment. It is upon precisely

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$4\frac{1}{2}$	$3\frac{1}{2}$	3	4	$2\frac{1}{2}$	2
$3\frac{1}{2}$	$2\frac{1}{2}$	4	3	2	$4\frac{1}{2}$
2	3	$2\frac{1}{2}$	$4\frac{1}{2}$	$3\frac{1}{2}$	4
$2\frac{1}{2}$	4	2	$3\frac{1}{2}$	$4\frac{1}{2}$	3
3	$4\frac{1}{2}$	$3\frac{1}{2}$	2	4	$2\frac{1}{2}$
4	2	$4\frac{1}{2}$	$2\frac{1}{2}$	3	$3\frac{1}{2}$

this expected variation in our estimate, the nature of which can be predicted when the errors by which the observed values are affected are a true random sample of the errors which contribute to our estimate, that the test of significance employed, namely the *Z* test of Fisher (5, p.190 *et seq.*) is based.

$4\frac{1}{2}$	$3\frac{1}{2}$	3	4	$2\frac{1}{2}$	2
D A B	A B D	B D A	D B A	B D A	B D A
$3\frac{1}{2}$	$2\frac{1}{2}$	4	3	2	$4\frac{1}{2}$
D B A	D B A	B D A	D B A	B D A	B D A
2	3	$2\frac{1}{2}$	$4\frac{1}{2}$	$3\frac{1}{2}$	4
B D A	A B D	A B D	B D A	D B A	A D B
$2\frac{1}{2}$	4	2	$3\frac{1}{2}$	$4\frac{1}{2}$	3
B D A	B A D	B A D	B D A	B A D	D A B
3	$4\frac{1}{2}$	$3\frac{1}{2}$	2	4	$2\frac{1}{2}$
A B D	B D A	A B D	B A D	A D B	D B A
4	2	$4\frac{1}{2}$	$2\frac{1}{2}$	3	$3\frac{1}{2}$
D A B	A D B	A B D	B D A	A D B	A B D

FIG. 2. Above: Random distribution of rates of seeding in a 6 by 6 Latin square. Below: Completed random arrangement for three varieties. Rates of seeding in bu. per acre. A = Abundance, B = Banner, D = Daubeney.

The random arrangement of the plots ensures that the estimate of error obtained is a valid one. It is however possible to utilize the fact, already noted, that the yield of neighboring plots is usually positively correlated, to eliminate from our comparisons certain major elements of soil heterogeneity. The result is a much reduced, but still accurately estimated, experimental error. This is accomplished by imposing certain restrictions upon the purely random arrangement. According to the particular restrictions employed, two types of arrangement are obtained, which have been designated by Fisher "Randomised Blocks" and the "Latin Square", respectively.

In the former, the experimental area is divided into a number of "blocks" of land, there being as many such blocks as there are to be replications. Each block consists of as many plots as there are treatments, varieties, etc., to be compared. The plots are then allotted at random to the various treatments, subject to the restriction that each treatment may occur once, and once only, in each block. By this arrangement the variations in soil fertility which may

affect our comparisons are confined to such as occur *within* blocks; the random arrangement of the plots in each block allowing the error thus introduced to be properly estimated. Differences in general fertility level between blocks are thus eliminated in the field, and, by the statistical procedure known as the Analysis of Variance (5, p. 190 *et seq.*) may also be eliminated from our estimate of error. There thus often results an experiment of considerably enhanced precision.

A further extension of the principle of restricted random arrangement, or Local Control as Fisher has termed it, leads to the Latin Square. Here there are as many replicates as there are treatments, the plots being arranged in the form of a square. The various treatments are again distributed at random over the plots, subject however to the restriction that each treatment shall occur once only in each horizontal row and each vertical column of plots forming the square. (An illustration of such an arrangement appears in the upper part of Fig. 2). In consequence, it is possible to eliminate soil differences both between rows and between columns of plots (*i.e.*, in two directions at right angles). At the same time a valid estimate of the remaining errors is obtained. When the number of comparisons to be made is between four and seven, this method will usually produce a very precise experiment. In practice, the plots need not of course be actually square, but may be varied in shape to suit the requirements of cultivation. Precision is however sometimes lost if they are made too long and narrow.

The foregoing brief exposition is intended merely as a summary of the main principles involved in these methods of field experimentation. Those interested are referred to References (4) and (5) for a more detailed discussion of these points. Examples of the practical application of both Randomised Block and Latin Square arrangements, and also of the methods of computation most advantageously employed in dealing with the results, will be found in (6), (7), (10) and (18). Proofs of some of the mathematical theorems underlying the Analysis of Variance will be found in (11).

Interesting evidence in support of Fisher's views has been provided by Tedin (17) as a result of comparing the estimate of error obtained when randomly determined Latin Squares and also systematic arrangements in a square were superimposed upon the plot yields of uniformity trials reported in the literature.

4. Consideration of the Present Arrangement in the Light of these Principles

While the principles discussed in the foregoing section were put forward after the present experiment had been done, it may be instructive to consider in their light the field arrangement used. It is at once obvious that this does not fulfil the conditions set forth above. In the first place, the systematic alternation of the "check" and "rate" plots may have some feature in common with variations in soil fertility, thus rendering the comparisons subject to unknown errors. The same objection, moreover, applies to the grouping of all the plots of any one seed rate together. As a possible consequence of this

arrangement, we might get, say, the comparison " $2\frac{1}{2}$ bushels *versus* check" being made on land of a quite different level of fertility from that employed for the comparison " $3\frac{1}{2}$ bushels *versus* check". Then we have only to make the not altogether unwarranted assumption that the optimum rate of seeding is to some extent dependent on fertility, to see that our two sets of observations may not be truly comparable, and that if they are not, no amount of check plots will make them so.

For example, the comparison " $2\frac{1}{2}$ bushels *versus* 3 (check)" might be made on land such that the average yield of the three-bushel plots was 50 bushels per acre. Here, owing to the limited capacity of the soil to support a crop, and the consequent mutually detrimental competition of plants densely spaced, the plots sown at the lower rate might give the higher total yield. On the other hand, the comparison " $3\frac{1}{2}$ bushels *versus* 3" might be made on more fertile land, where the average yield of the three-bushel plots was, say, 75 bushels per acre. In this case, as a result of the more favorable conditions, the higher rate of seeding might well give a significantly higher return. The incautious might thus be led to assume that greater yields were to be obtained by seeding either above or below the three-bushel rate, a minimum return being obtained from seed rates in the neighborhood of three bushels. In fact, of course, the observed effect would be entirely spurious.

Extreme though this example may appear, we shall have cause to see, upon examination of the data, that situations of a similar nature involving differences in fertility level of quite the same order, did in fact occur.

Similar considerations apply to the segregation of all the plots of one variety upon one "range", or part, of the experimental block of land, with the added fact that here we have no means of determining to what extent soil differences between ranges may have entered as a disturbing factor.

A word must also be said concerning the rates of seeding adopted for the different varieties. It will be observed that these vary from 1 to 3 bushels in the case of Daubeney, from 2 to 4 in the case of Banner, and from $2\frac{1}{2}$ to $4\frac{1}{2}$ in the case of Abundance. The lowest rate at which Abundance was sown is thus only $\frac{1}{2}$ bushel less than the highest rate of Daubeney. This was done in order to centre the various rates employed for each variety upon the rate at which that variety was most commonly sown in agricultural practice. Since, however, one of the objects of the experiment was to ascertain whether varieties differing in seed and plant size and in tillering capacity should be sown at the same, or different, rates in variety trials, it would appear that a better arrangement would have been to apply a suitably extended series of seed rates impartially to all three varieties.

It may be of some interest to illustrate the application of the principles of Section 3 by putting forward an alternative scheme to that here used. This may best be done by employing the doubly restricted random arrangement, or Latin Square.

The first point to be noted is that by discarding the check plot system the major proportion of the 99 check plots which it necessitated are eliminated. This makes it possible to extend the range of seed rates, whilst still keeping the

expenditure of land, labor, and materials considerably below that required by the check plot system. We may therefore decide to compare the behavior of all three varieties when sown at 2, $2\frac{1}{2}$, 3, $3\frac{1}{2}$, 4 and $4\frac{1}{2}$ bushels per acre.

Six replicate plots of each of these seed rates are then arranged in a randomly determined Latin Square, as shown in the upper portion of Fig. 2. Each of the plots there represented by a square would actually consist of 27 drill rows $18\frac{1}{2}$ ft. long, spaced 7 in. apart; *i.e.*, three of our nine-row plots laid side by side. These plots are now each divided into three sub-plots of nine drill-rows, and to the three sub-plots in each rate-plot the three experimental varieties are allotted at random. The completed arrangement is shown in the lower portion of Fig. 2.

The restricted random arrangement allows soil differences between the rows and columns of large rate-plots to be eliminated, and at the same time makes possible a valid estimate of the remaining errors to which our comparisons are subject. The procedure of growing the three varieties in close proximity to each other in all the rate-plots allows attention to be concentrated on what is after all the main point at issue: namely, the differential response of these varieties to variations in the rate of seeding.

The centre row of each nine-row plot could be used for the determination of stand and tillering, although this method of sampling is by no means an ideal one. Discarding the two outside rows then leaves six rows from which to determine the yield. At harvest time one foot would of course be removed from each end of the rows, as before.

The total number of nine-row plots in this arrangement is 108. In the arrangement actually used it was 195. It thus appears that two Latin Squares, giving in all 12 plots of each kind, could be laid down with little more labor than was involved in the experiment as actually carried out. This should produce a rather precise experiment, the results of which could be accepted with some confidence. By reducing the size of the plots from nine to seven rows, which would mean the determination of yield from four rows, the labor requirements could be brought below those of the arrangement actually adopted. This would of course be achieved at the expense of a certain loss of accuracy.

Each Latin Square used should of course be a separately determined random arrangement.

5. Analysis of the Results

A summary of the observational data is given in Tables I, II, III and IV¹. This includes determinations of the weight per 1000 kernels and percentage germination of the seed used, the number of germinable seeds sown, and the mean values found at each seed rate for stand of plants, number of tillers and yield of grain. The mean values of observations made upon adjacent "check" and "rate" plots (see Fig. 1) appear on the same line.

Upon an examination of these, it is at once apparent that the differences in yield between the various check and rate plots are surprisingly small, being

¹The complete observational data are too voluminous to be practicable of publication, but are available for study in the Library of the National Research Council of Canada.

TABLE I
MEAN VALUES OF OBSERVATIONAL DATA, BY YEARS, VARIETIES AND RATES.
DOMINION EXPERIMENTAL STATION, CHARLOTTETOWN, P.E.I.

Year	Variety	Weight of 1000 kernels, gm.	Germination, %	Rate of seeding per acre (bushels)		Number of germinable seeds per acre (thousands)		Number of plants per acre (thousands)		Number of tillers per acre (thousands)		Stand of plants in % of germinable seeds sown		Number of tillers per plant		Yield of grain per acre (bushels)	
				Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate
1924	Abundance	39.7	100	3	2½	1159	969	960	772	987	820	82.5	79.7	1.03	1.06	40.65	39.61
				3	3½	1159	1353	982	1084	1023	1124	84.8	80.2	1.04	1.04	33.03	36.23
				3	4	1159	1548	953	1125	980	1148	82.2	72.7	1.03	1.02	31.95	32.83
				3	4½	1159	1742	1000	1429	1064	1465	86.3	82.0	1.06	1.02	46.55	48.90
	Banner	34.4	100	3	2	1335	892	1183	782	1217	818	88.6	87.8	1.03	1.04	47.51	45.16
				3	2½	1335	1113	1214	1019	1249	1059	90.9	91.7	1.03	1.04	53.33	51.70
				3	3½	1335	1557	1202	1375	1232	1402	90.0	88.3	1.03	1.02	50.43	50.37
				3	4	1335	1783	1161	1556	1204	1585	87.0	87.3	1.04	1.02	64.53	65.69
	Dauboney	21.2	100	2	1	1448	724	1203	621	1500	956	83.3	85.7	1.24	1.54	33.08	28.08
				2	1½	1448	1086	1206	955	1574	1419	83.2	88.0	1.30	1.49	30.96	28.39
				2	2½	1448	1810	1252	1563	1543	1839	86.4	86.3	1.23	1.18	33.06	34.53
				2	3	1448	2172	1263	1783	1883	2119	87.2	82.1	1.49	1.20	40.84	47.34
1925	Abundance	32.2	100	3	2½	1431	1193	1203	971	1402	1226	83.7	81.4	1.16	1.25	35.91	36.03
				3	3½	1431	1670	1163	1331	1306	1552	81.3	79.7	1.13	1.15	44.58	48.07
				3	4	1431	1908	1166	1553	1340	1760	82.4	80.4	1.14	1.13	52.54	54.66
				3	4½	1431	2147	1205	1628	1324	2008	84.2	75.8	1.17	1.24	45.26	47.62
	Banner	30.1	100	3	2	1530	1020	1053	708	1274	859	68.8	69.4	1.21	1.22	42.80	30.96
				3	2½	1530	1275	1153	894	1438	1164	75.3	70.1	1.25	1.31	43.22	41.08
				3	3½	1530	1785	1139	1275	1398	1701	74.4	71.4	1.23	1.24	44.74	46.38
				3	4	1530	2040	1174	1428	1389	1726	76.7	69.4	1.18	1.21	42.20	44.14
	Dauboney	20.7	100	2	1	1482	741	1019	522	1339	819	68.7	69.6	1.32	1.56	33.20	23.94
				2	1½	1482	1111	1120	738	1435	1121	75.6	68.9	1.29	1.52	33.13	24.84
				2	2½	1482	1852	995	1158	1625	1712	67.1	69.1	1.60	1.51	34.63	39.01
				2	3	1482	2223	991	1334	1695	2050	66.9	60.0	1.80	1.58	38.14	40.41
1926	Abundance	33.6	95	3	2½	1303	1086	1087	817	1103	832	82.7	75.2	1.02	1.63	26.04	25.32
				3	3½	1303	1521	1050	1252	1073	1275	80.5	82.3	1.02	1.02	39.18	39.05
				3	4	1303	1738	1067	1344	1095	1363	81.8	77.3	1.03	1.02	43.91	45.08
				3	4½	1303	1946	1062	1380	1076	1411	81.5	70.9	1.04	1.02	40.83	44.40
	Banner	33.6	99	3	2	1362	910	1004	674	1018	700	78.3	74.0	1.08	1.04	44.56	42.39
				3	2½	1362	1136	1092	849	1112	860	80.5	74.8	1.01	1.01	47.32	46.64
				3	3½	1362	1559	985	1209	945	1226	72.3	72.1	1.08	1.01	56.90	56.53
				3	4	1362	1815	1058	1306	1090	1330	77.6	76.0	1.03	1.02	62.04	62.21
	Dauboney	25.2	96	2	1	1177	588	856	363	1594	941	72.7	61.6	1.88	2.61	64.95	56.98
				2	1½	1177	883	853	645	1382	1306	73.2	73.1	1.72	1.83	51.57	49.24
				2	2½	1177	1466	886	1014	1506	1562	75.3	69.2	1.71	1.60	57.57	56.91
				2	3	1177	1761	926	1247	1719	1826	78.7	70.9	1.77	1.46	61.24	64.37

TABLE II
MEAN VALUES OF OBSERVATIONAL DATA, BY YEARS, VARIETIES AND RATES.
MACDONALD COLLEGE, STE. ANNE DE BELLEVUE, QUEBEC

Year	Variety	Weight of 1000 kernels, gm.	Germination, %	Rate of seeding per acre (bushels)		Number of germinable seeds per acre (thousands)		Number of plants per acre (thousands)		Number of tillers per acre (thousands)		Stand of plants in % of germinable seeds sown		Number of tillers per plant		Yield of grain per acre (bushels)	
				Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate
1924	Abundance	39.0	99	3	2½	1175	979	617	541	785	723	52.5	55.2	1.4	1.6	52.34	54.34
				3	3½	1175	1371	770	990	914	1129	65.5	72.2	1.2	1.1	43.71	43.86
				3	4	1175	1567	579	803	738	941	42.3	51.3	1.3	1.2	41.62	43.83
				3	4½	1175	1762	462	834	620	992	39.3	47.3	1.3	1.2	52.02	54.04
	Banner	34.2	99	3	2	1340	893	523	357	646	478	39.1	39.9	1.2	1.4	49.52	49.64
				3	2½	1340	1116	912	696	1017	788	68.1	62.3	1.1	1.1	54.98	54.34
				3	3½	1340	1563	811	877	903	997	60.5	56.1	1.1	1.1	52.58	55.72
				3	4	1340	1786	602	823	731	1001	44.9	46.1	1.2	1.3	51.81	52.49
	Daubency	22.8	98	2	1	1376	663	480	312	795	604	36.2	47.1	1.7	2.2	42.27	41.80
				2	1½	1376	995	899	684	1095	889	67.8	75.0	1.2	1.2	37.22	38.07
				2	2½	1376	1658	718	986	978	1212	54.1	59.5	1.3	1.2	36.46	38.04
				2	3	1376	1990	596	1108	876	1404	44.9	55.7	1.5	1.3	50.93	46.65
1925	Banner	29.3	98	3	2	1549	1033	1068	847	1241	958	68.9	82.0	1.1	1.1	60.90	57.28
				3	2½	1549	1291	993	862	1302	1154	63.0	66.8	1.3	1.3	72.07	69.73
				3	3½	1549	1807	871	974	1229	1299	56.3	53.9	1.4	1.3	70.86	69.81
				3	4	1549	2065	859	935	1182	1469	55.5	45.3	1.3	1.4	72.51	73.21
	Daubency	23.0	96	2	1	1288	644	923	564	1471	1059	71.7	87.6	1.6	1.8	58.78	50.26
				2	1½	1288	966	966	724	1447	1381	70.4	74.8	1.6	1.9	62.91	62.19
				2	2½	1288	1610	999	1092	1646	1689	77.6	68.1	1.6	1.5	68.00	68.51
				2	3	1288	1932	1026	1078	1734	1966	79.7	55.8	1.7	1.8	64.82	65.02
1926	Abundance	33.0	95	3	2½	1362	1135	755	730	1054	1006	55.5	64.3	1.4	1.4	58.68	59.47
				3	3½	1362	1589	755	945	1056	1241	55.4	59.4	1.4	1.3	59.19	62.68
				3	4	1362	1816	786	1022	1146	1454	57.8	56.3	1.4	1.4	66.53	65.74
				3	4½	1362	2045	823	1035	1138	1557	60.5	50.6	1.4	1.5	64.53	65.09
	Banner	33.6	99	3	2	1366	910	723	501	1140	890	53.1	55.1	1.6	1.8	72.85	71.76
				3	2½	1366	1138	1039	706	1166	1028	76.1	62.0	1.1	1.4	74.08	75.77
				3	3½	1366	1593	1006	787	1093	1252	73.7	49.4	1.1	1.6	74.69	73.88
				3	4	1366	1821	786	782	1089	1304	57.6	43.0	1.4	1.7	71.61	72.84
	Daubency	25.2	96	2	1	1223	589	787	456	1075	865	64.4	77.5	1.4	1.9	47.52	41.84
				2	1½	1223	920	676	553	1240	1343	55.3	60.2	1.8	2.4	75.03	72.70
				2	2½	1223	1534	689	827	1280	1401	56.4	54.0	1.8	1.9	68.27	69.98
				2	3	1223	1840	633	838	1272	1431	51.8	45.6	2.0	1.7	64.49	66.68

Year	Variety	Weight of 1000 kernels, gm.	Germination, %	Rate of seeding per acre (bushels)		Number of germinable seeds per acre (thousands)		Number of plants per acre (thousands)		Number of tillers per acre (thousands)		Stand of plants in % of germinable seeds sown		Number of tillers per plant		Yield of grain per acre (bushels)	
				Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate
1924	Abundance	39.0	96	3	2½	1140	946	978	858	997	870	85.3	89.8	1.02	1.01	25.77	27.14
				3	3½	1140	1334	987	1220	1000	1226	86.5	91.6	1.01	1.00	28.47	27.14
				3	4	1140	1516	1022	1335	1044	1295	89.5	88.1	1.01	1.00	24.92	22.17
				3	4½	1140	1602	1006	1387	1009	1387	88.2	86.3	1.00	1.00	17.43	15.67
	Banner	34.2	100	3	2	1353	901	1153	763	1192	868	91.7	84.5	1.03	1.12	37.11	36.17
				3	2½	1353	1127	1120	948	1123	972	82.5	83.7	1.01	1.03	32.66	34.54
				3	3½	1353	1579	1117	1292	1126	1296	82.5	81.7	1.01	1.00	25.07	22.12
				3	4	1353	1801	1139	1502	1148	1506	84.1	83.2	1.01	1.00	21.86	21.76
	Daubney	22.8	95	2	1	1285	643	1144	556	1190	775	88.8	86.6	1.04	1.39	32.38	29.79
				2	1½	1285	964	1114	873	1136	914	86.5	90.4	1.02	1.05	15.10	15.86
				2	2½	1285	1607	1145	1351	1161	1366	89.1	84.1	1.01	1.01	15.86	13.76
				2	3	1285	1928	1161	1582	1175	1588	90.5	82.1	1.01	1.00	12.34	10.85
1925	Abundance	36.4	98	3	2½	1247	1039	1130	945	1151	1071	89.6	91.0	1.03	1.03	82.84	80.37
				3	3½	1247	1454	1150	1250	1211	1384	92.2	86.0	1.12	1.14	87.28	90.66
				3	4	1247	1662	1180	1552	1224	1557	94.6	93.4	1.04	1.01	83.25	84.61
				3	4½	1247	1870	1129	1714	1211	1702	90.5	91.7	1.07	1.02	90.20	88.86
	Banner	30.8	98	3	2	1471	981	1359	938	1404	1069	92.4	95.6	1.05	1.14	88.10	86.16
				3	2½	1471	1226	1367	1156	1333	1173	92.4	94.3	1.08	1.05	88.97	95.05
				3	3½	1471	1716	1376	1628	1350	1565	93.6	95.0	1.00	1.00	86.90	84.83
				3	4	1471	1962	1372	1862	1326	1764	93.2	95.0	1.00	1.00	91.43	86.39
	Daubney	21.9	96	2	1	1355	677	1267	622	1393	1458	93.6	91.8	1.15	1.34	72.04	71.08
				2	1½	1355	1016	1277	932	1575	1477	94.3	91.7	1.23	1.52	72.64	69.64
				2	2½	1355	1693	1255	1547	1619	1502	92.7	91.3	1.28	1.06	72.30	69.70
				2	3	1355	2032	1254	1985	1492	1869	92.6	97.7	1.19	1.00	70.83	70.00
1926	Abundance	33.6	95	3	2½	1306	1088	982	837	1187	960	75.2	76.9	1.21	1.15	38.75	40.09
				3	3½	1306	1524	942	1121	1167	1318	72.1	73.6	1.25	1.18	36.96	37.82
				3	4	1306	1742	951	1314	1168	1336	72.8	75.3	1.32	1.17	33.84	32.97
				3	4½	1306	1959	1002	1438	1323	1969	76.7	73.4	1.32	1.37	35.00	29.37
	Banner	33.6	99	3	2	1365	910	974	691	1194	852	71.4	76.0	1.23	1.23	54.60	54.79
				3	2½	1365	1138	924	809	1120	1022	67.7	71.1	1.21	1.27	56.68	57.89
				3	3½	1365	1593	913	1008	1099	1178	66.9	63.3	1.21	1.17	47.24	46.70
				3	4	1365	1820	967	1343	1232	1483	70.8	73.8	1.28	1.11	45.33	43.12
	Daubney	25.2	96	2	1	1177	589	809	383	1010	800	68.7	65.1	1.25	2.09	35.85	34.89
				2	1½	1177	883	766	625	940	863	65.1	70.7	1.23	1.38	34.35	33.32
				2	2½	1177	1472	701	1036	1011	1126	59.4	70.4	1.44	1.09	36.33	37.56
				2	3	1177	1766	762	1244	1000	1376	64.7	70.4	1.31	1.11	31.27	30.33

TABLE IV
MEAN VALUES OF OBSERVATIONAL DATA, BY YEARS, VARIETIES AND RATES.
UNIVERSITY OF ALBERTA, EDMONTON, ALBERTA

Year	Variety	Weight of 1000 kernels, gm.	Germination, %	Rate of seeding per acre (bushels)		Number of germinable seeds per acre (thousands)		Number of plants per acre (thousands)		Number of tillers per acre (thousands)		Stand of plants in % of germinable seeds sown		Number of tillers per plant		Yield of grain per acre (bushels)	
				Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate	Check	Rate
1924	Abundance	39.0	96	3	2 1/2	1131	941	982	837	1155	1060	86.8	88.9	1.18	1.30	92.10	84.92
				3	3 1/2	1131	1372	983	1092	1095	1232	86.9	82.6	1.11	1.13	84.82	80.82
				3	4 1/2	1131	1512	977	1303	1160	1421	86.4	86.2	1.19	1.09	90.03	94.12
				3	4 1/2	1131	1697	1028	1471	1295	1363	90.9	86.7	1.26	1.06	112.04	110.80
	Banner	34.2	100	3	2	1344	896	1280	1113	1308	1150	95.2	124.2	1.02	1.04	122.61	129.45
				3	2 1/2	1344	1122	1202	1163	1211	1183	89.4	103.6	1.01	1.02	124.71	126.89
				3	3 1/2	1344	1570	1299	1386	1307	1393	87.7	88.4	1.01	1.01	133.12	129.75
				3	4	1344	1792	1180	1384	1185	1383	87.8	77.2	1.00	1.00	124.35	120.70
	Daubney	22.8	95	2	1	1276	638	1135	657	2535	2425	89.0	102.9	2.26	3.74	87.71	81.44
				2	1 1/2	1276	959	1171	872	2389	2429	89.8	92.9	2.08	2.79	84.56	81.35
				2	2 1/2	1276	1598	1240	1311	2634	2630	87.1	82.0	2.04	2.02	78.82	79.90
				2	3	1276	1919	1145	1182	2356	2202	89.7	61.6	2.07	1.89	86.32	86.46
1925	Abundance	28.7	99	3	2 1/2	1589	1322	1285	1066	1313	1111	80.9	80.7	1.02	1.04	94.22	99.67
				3	3 1/2	1589	1851	1305	1513	1310	1317	82.1	81.4	1.00	1.00	80.32	91.73
				3	4	1589	2116	1289	1724	1305	1732	81.1	81.4	1.01	1.00	91.38	97.46
				3	4 1/2	1589	2381	1338	1942	1364	1960	84.2	81.6	1.02	1.01	99.36	95.31
	Banner	29.1	98	3	2	1548	1032	1324	875	1340	1018	85.5	84.8	1.01	1.16	123.55	120.60
				3	2 1/2	1548	1290	1315	1095	1348	1159	85.0	84.9	1.03	1.06	119.00	127.02
				3	3 1/2	1548	1806	1350	1547	1356	1551	87.2	85.7	1.00	1.00	116.93	116.17
				3	4	1548	2068	1328	1761	1340	1763	85.8	85.1	1.01	1.00	114.15	110.92
	Daubney	21.1	96	2	1	1394	697	1102	574	1590	1358	79.1	82.4	1.44	2.36	101.67	94.37
				2	1 1/2	1394	1045	1193	911	1646	1536	85.6	87.2	1.38	1.70	106.95	101.73
				2	2 1/2	1394	1747	1215	1547	1622	1795	87.1	86.7	1.34	1.16	129.97	102.91
				2	3	1394	2095	1107	1607	1576	1830	79.4	76.7	1.42	1.14	105.18	106.77
1926	Abundance	33.5	95	3	2 1/2	1303	1086	1112	1027	1116	1037	85.3	94.6	1.00	1.01	89.02	88.56
				3	3 1/2	1303	1521	1146	1374	1157	1378	87.7	80.4	1.01	1.00	78.14	75.58
				3	4	1303	1742	1182	1509	1188	1513	90.7	86.6	1.01	1.00	89.17	80.06
				3	4 1/2	1303	1935	1126	1540	1140	1347	86.4	78.8	1.01	1.00	89.51	88.14
	Banner	35.5	99	3	2	1285	855	1082	748	1087	795	84.2	87.4	1.00	1.06	84.38	82.46
				3	2 1/2	1285	1068	1135	922	959	959	86.1	86.3	1.02	1.04	84.30	95.17
				3	3 1/2	1285	1496	1177	1230	1182	1247	91.6	82.1	1.01	1.00	83.48	87.59
				3	4	1285	1711	1156	1352	1170	1354	90.0	79.0	1.01	1.00	82.68	99.11
	Daubney	25.5	96	2	1	1154	579	991	530	1427	1310	85.9	91.4	1.45	2.48	68.67	65.43
				2	1 1/2	1154	864	993	984	1501	1358	76.3	79.1	1.66	1.95	73.88	73.34
				2	2 1/2	1154	1448	889	1070	1279	1164	77.0	77.2	1.44	1.20	67.04	68.35
				2	3	1154	1733	924	1358	1245	1488	80.0	78.3	1.35	1.10	69.12	73.31

often only of the order of a bushel or so per acre. The first step in the analysis would therefore appear to be to ascertain whether in fact the differences in seed rate do appear to have influenced yield to any significant extent, or whether such variations as have occurred might reasonably be regarded as purely chance effects. This may be done in the following manner: from the sum of the yields of the two three-row plots constituting each large rate-plot we may subtract the yields of the two three-row check plots immediately adjacent thereto (see Fig. 1). In this way, a set of eight differences is obtained for each comparison. To test if the mean of these eight differences departs significantly from zero we calculate the quantity

$$t = \frac{\bar{x} \sqrt{n^1(n^1-1)}}{\sqrt{S(x-\bar{x})^2}}$$

Where \bar{x} is the mean of the eight observed differences, S indicates summation over the eight individuals, and $n^1=8$. The actual process of calculation is simplified by noting that

$$\frac{\bar{x} \sqrt{n^1(n^1-1)}}{\sqrt{S(x-\bar{x})^2}} = \frac{S(x)}{\sqrt{S(x-\bar{x})^2}} \cdot \frac{\sqrt{n^1-1}}{\sqrt{n^1}}$$

This procedure was applied to the whole of the observed grain yields, with the results shown in Table V. Owing to shortage of seed, gnawing of sheaves by mice, and various other causes, the number of plots from which yields were available was in some cases reduced. The second column of the table shows the number of differences it was possible to form in these cases. The fourth column gives the sum of the squares of the deviations of the observed differences from their mean, and the fifth column the values of the ratio t .

Now the distribution of the statistic t is known in the case when our two sets of observations are random samples from a homogeneous normal population, and the probability that any value of t obtained should lie between certain fixed limits may be found from a table of "Student's" integral (5, p.139). In making tests of significance upon isolated samples, therefore, it is customary to ascertain from the table what is the probability of obtaining by chance a value of t equal to or greater than that actually observed. If this probability is sufficiently small, we then say with some confidence that the observed effects are not of a purely random nature, but that the various treatments, etc., employed have significantly influenced the results. Values having a probability of 0.05 or less are usually regarded as significant in agricultural experimentation. Such values have been marked with an asterisk (*) in Table V.

In the present case, however, we may carry our test of significance one stage further. The total number of values of t in Table V, based on seven degrees of freedom ($n^1=8$) is 113. From the table of t we may determine the proportion of these expected to fall between different limits. Then, with this "expected" distribution we may compare the distribution actually obtained. This has been done in Table VI.

TABLE V

RATIO OF OBSERVED DIFFERENCES IN YIELD TO THEIR ESTIMATED STANDARD ERROR

Rate of seeding comparison			n^1	$S(x)$	$S(x-\bar{x})^2$	t
Charlottetown						
1924	Abundance	2½ bu. v. 3 bu.	8	-16.70	294.70	0.915
		3½ bu. v. 3 bu.	8	51.08	291.67	2.798*
		4 bu. v. 3 bu.	8	14.08	98.75	1.325
		4½ bu. v. 3 bu.	7	30.27	215.46	1.909
	Banner	2 bu. v. 3 bu.	8	-37.59	648.32	1.381
		2½ bu. v. 3 bu.	8	-26.14	446.94	1.157
		3½ bu. v. 3 bu.	8	2.18	255.91	0.128
		4 bu. v. 3 bu.	7	10.39	729.64	0.356
	Daubeney	1 bu. v. 2 bu.	8	-79.97	210.70	5.142*
		1½ bu. v. 2 bu.	8	-41.16	74.49	4.460*
		2½ bu. v. 2 bu.	8	23.66	190.06	1.606
		3 bu. v. 2 bu.	7	96.84	312.88	5.068*
1925	Abundance	2½ bu. v. 3 bu.	7	13.83	382.32	0.655
		3½ bu. v. 3 bu.	7	57.38	1010.43	1.671
		4 bu. v. 3 bu.	8	34.06	1055.02	0.981
		4½ bu. v. 3 bu.	8	37.93	880.02	1.196
	Banner	2 bu. v. 3 bu.	8	-189.54	1166.66	5.191*
		2½ bu. v. 3 bu.	8	-34.27	667.84	1.240
		3½ bu. v. 3 bu.	8	33.63	3567.85	0.527
		4 bu. v. 3 bu.	8	30.98	1267.17	1.814
	Daubeney	1 bu. v. 2 bu.	8	-148.15	533.21	6.001*
		1½ bu. v. 2 bu.	8	-132.69	700.19	4.691*
		2½ bu. v. 2 bu.	8	70.16	1110.17	1.970
		3 bu. v. 2 bu.	8	36.09	380.13	1.732
1926	Abundance	2½ bu. v. 3 bu.	8	-11.41	115.56	0.993
		3½ bu. v. 3 bu.	8	-2.04	107.02	0.184
		4 bu. v. 3 bu.	8	18.73	122.16	1.585
		4½ bu. v. 3 bu.	8	57.09	269.82	3.251*
	Banner	2 bu. v. 3 bu.	8	-34.69	210.00	2.239
		2½ bu. v. 3 bu.	8	-10.89	1251.21	0.288
		3½ bu. v. 3 bu.	8	-5.93	386.15	0.282
		4 bu. v. 3 bu.	8	2.69	102.76	0.248
	Daubeney	1 bu. v. 2 bu.	8	-127.44	2201.05	2.541*
		1½ bu. v. 2 bu.	8	-37.15	702.33	1.311
		2½ bu. v. 2 bu.	8	-10.51	840.81	0.339
		3 bu. v. 2 bu.	8	48.94	689.54	1.743
Macdonald College						
1924	Abundance	2½ bu. v. 3 bu.	8	31.95	366.48	1.561
		3½ bu. v. 3 bu.	8	2.35	64.03	0.274
		4 bu. v. 3 bu.	8	34.95	549.47	1.394
		4½ bu. v. 3 bu.	8	32.40	684.89	1.158
	Banner	2 bu. v. 3 bu.	8	1.87	677.81	0.067
		2½ bu. v. 3 bu.	8	-10.31	282.99	0.573
		3½ bu. v. 3 bu.	8	50.22	838.96	1.634
		4 bu. v. 3 bu.	8	10.78	213.37	0.690
	Daubeney	1 bu. v. 2 bu.	8	-7.42	494.23	0.312
		1½ bu. v. 2 bu.	8	13.72	123.70	1.154
		2½ bu. v. 2 bu.	8	25.35	301.13	1.366
		3 bu. v. 2 bu.	8	-68.48	272.01	3.884

TABLE V—Continued

Rate of seeding comparison			n^1	$S(x)$	$S(x-\bar{x})^2$	t
1925	Banner	2 bu. v. 3 bu.	8	-57.90	923.76	1.782
		2½ bu. v. 3 bu.	8	-51.84	1021.07	1.518
		3½ bu. v. 3 bu.	8	-16.84	329.45	0.868
		4 bu. v. 3 bu.	8	11.23	380.08	0.520
	Daubeney	1 bu. v. 2 bu.	8	-136.40	527.49	5.555*
		1½ bu. v. 2 bu.	8	-11.56	259.26	0.672
		2½ bu. v. 2 bu.	8	8.28	323.81	0.431
		3 bu. v. 2 bu.	8	3.26	479.46	0.139
	Abundance	2½ bu. v. 3 bu.	5	14.14	660.23	0.492
		3½ bu. v. 3 bu.	8	55.82	326.94	2.888*
		4 bu. v. 3 bu.	8	-12.65	1418.28	0.314
		4½ bu. v. 3 bu.	8	8.96	207.22	0.586
Scott, 1924	Banner	2 bu. v. 3 bu.	3	-6.49	0.91	5.544*
		2½ bu. v. 3 bu.	8	27.00	569.26	1.058
		3½ bu. v. 3 bu.	8	-12.97	252.92	0.763
		4 bu. v. 3 bu.	8	19.76	333.21	1.012
	Daubeney	1 bu. v. 2 bu.	8	-90.84	120.60	7.738*
		1½ bu. v. 2 bu.	8	-37.38	370.58	1.816
		2½ bu. v. 2 bu.	8	27.43	120.64	2.336
		3 bu. v. 2 bu.	8	35.03	490.34	1.480
	Abundance	2½ bu. v. 3 bu.	8	21.82	120.51	1.859
		3½ bu. v. 3 bu.	8	-21.43	234.06	1.310
		4 bu. v. 3 bu.	7	-38.34	431.52	1.709
		4½ bu. v. 3 bu.	6	-28.23	95.05	2.643*
	Banner	2 bu. v. 3 bu.	8	-14.95	302.38	0.804
		2½ bu. v. 3 bu.	8	29.92	302.25	1.610
		3½ bu. v. 3 bu.	7	-41.41	409.38	1.895
		4 bu. v. 3 bu.	7	-10.41	167.61	0.744
	Daubeney	1 bu. v. 2 bu.	8	-41.53	554.34	1.469
		1½ bu. v. 2 bu.	8	12.12	38.65	1.824
		2½ bu. v. 2 bu.	8	-33.30	191.22	2.252
		3 bu. v. 2 bu.	7	-13.23	103.90	1.202
1925	Abundance	2½ bu. v. 3 bu.	7	-8.88	1006.68	0.259
		3½ bu. v. 3 bu.	8	54.08	1059.66	1.554
		4 bu. v. 3 bu.	8	21.83	1017.87	0.636
		4½ bu. v. 3 bu.	7	-27.66	1514.38	0.658
	Banner	2 bu. v. 3 bu.	8	-30.91	617.54	1.164
		2½ bu. v. 3 bu.	8	97.22	1835.14	2.123
		3½ bu. v. 3 bu.	8	-33.14	1061.52	0.951
		4 bu. v. 3 bu.	7	-56.17	1600.57	1.300
	Daubeney	1 bu. v. 2 bu.	8	0.71	887.37	0.030
		1½ bu. v. 2 bu.	8	-48.17	708.75	1.692
		2½ bu. v. 2 bu.	8	-41.59	878.99	1.312
		3 bu. v. 2 bu.	7	-5.61	565.81	0.218
1926	Abundance	2½ bu. v. 3 bu.	8	21.21	262.39	1.225
		3½ bu. v. 3 bu.	8	13.53	120.11	1.155
		4 bu. v. 3 bu.	8	-18.82	146.81	1.453
		4½ bu. v. 3 bu.	7	-86.77	313.28	4.539*

TABLE V—Continued

Rate of seeding comparison			n^1	$S(x)$	$S(x-\bar{x})^2$	t	
1926	Banner	2 bu. v. 3 bu.	8	3.15	533.93	0.128	
		2½ bu. v. 3 bu.	8	19.44	472.81	0.836	
		3½ bu. v. 3 bu.	8	-8.64	839.56	0.279	
		4 bu. v. 3 bu.	7	-22.94	54.89	2.867*	
	Daubeney	1 bu. v. 2 bu.	8	-15.33	298.23	0.830	
		1½ bu. v. 2 bu.	8	-16.42	284.29	0.911	
		2½ bu. v. 2 bu.	8	19.64	1451.26	0.482	
		3 bu. v. 2 bu.	7	18.54	266.06	1.063	
	University of Alberta						
	1924	Abundance	2½ bu. v. 3 bu.	8	-114.14	1791.69	2.522*
			3½ bu. v. 3 bu.	8	-63.98	6303.02	0.754
			4 bu. v. 3 bu.	4	32.71	182.61	2.096
4½ bu. v. 3 bu.			4	-9.92	943.39	0.280	
Banner		2 bu. v. 3 bu.	6	101.67	1291.18	2.583*	
		2½ bu. v. 3 bu.	8	34.81	2058.27	0.717	
		3½ bu. v. 3 bu.	6	-42.86	3622.57	2.056	
		4 bu. v. 3 bu.	6	-40.75	834.21	1.288	
Daubeney		1 bu. v. 2 bu.	8	-100.31	164.56	7.315*	
		1½ bu. v. 2 bu.	8	-51.43	953.66	1.558	
		2½ bu. v. 2 bu.	8	17.32	1659.85	0.398	
		3 bu. v. 2 bu.	8	-3.96	3031.06	0.067	
1925	Abundance	2½ bu. v. 3 bu.	8	87.28	1774.69	1.938	
		3½ bu. v. 3 bu.	8	38.59	489.89	1.631	
		4 bu. v. 3 bu.	8	-225.90	1626.49	5.240*	
		4½ bu. v. 3 bu.	7	-51.44	2072.59	1.046	
	Banner	2 bu. v. 3 bu.	8	-47.10	811.77	1.546	
		2½ bu. v. 3 bu.	8	6.59	1236.38	0.175	
		3½ bu. v. 3 bu.	8	35.49	467.00	1.536	
		4 bu. v. 3 bu.	8	-51.70	55.65	6.483*	
	Daubeney	1 bu. v. 2 bu.	8	-116.80	1087.01	3.314*	
		1½ bu. v. 2 bu.	8	-70.77	355.61	3.510*	
		2½ bu. v. 2 bu.	8	46.91	909.92	1.455	
		3 bu. v. 2 bu.	8	22.65	310.30	1.190	
1926	Abundance	2½ bu. v. 3 bu.	8	-7.32	805.16	0.241	
		3½ bu. v. 3 bu.	8	-40.92	1311.30	1.057	
		4 bu. v. 3 bu.	8	-1.74	651.26	0.638	
		4½ bu. v. 3 bu.	8	-21.88	3624.45	0.340	
	Banner	2 bu. v. 3 bu.	8	-30.72	667.40	1.112	
		2½ bu. v. 3 bu.	8	61.98	1442.20	1.527	
		3½ bu. v. 3 bu.	8	-14.23	588.08	0.549	
		4 bu. v. 3 bu.	7	-51.47	570.32	1.995	
	Daubeney	1 bu. v. 2 bu.	8	-51.91	403.56	2.417*	
		1½ bu. v. 2 bu.	8	-24.62	556.42	0.976	
		2½ bu. v. 2 bu.	8	12.95	269.39	0.738	
		3 bu. v. 2 bu.	8	66.92	649.79	2.456*	

TABLE VI
DISTRIBUTION OF VALUES OF t

t	"Ex-pected"	Actual
0.000		
0.130	11.3	5
0.263	11.3	5
0.402	11.3	9
0.549	11.3	5
0.711	11.3	6
0.896	11.3	9
1.119	11.3	10
1.415	11.3	15
1.895	11.3	23
2.365	5.65	6
2.998	3.77	6
3.499	1.13	2
∞	1.13	12

The distribution of the values of t actually obtained is clearly not in accord with that expected as a result of purely random fluctuations in the yields of the various plots. There is a deficiency of the lower values and a notable excess of the higher ones. We therefore conclude that, taking the experiment as a whole, the plots sown at different rates have shown a significant tendency to differ in yield.

The individual "significant differences" in Table V are not very numerous, and are scattered throughout the table. It appeared that the method of *regression* might be used to advantage in examining more closely into the nature and extent of the associated variations in seed rate and yield. Before proceeding with this, however, it may be of some interest to consider briefly the quantities $S(x - \bar{x})^2$ of Table V. These furnish a direct measure of the degree of *precision* attained in the experiment at the various stations in different years.

By addition, a total sum of squares of deviations of observed differences from their respective means is obtained for each station in each year. On division by the appropriate number of degrees of freedom these give the mean squares of Table VII. Obviously, the nearer the eight differences belonging to each

comparison approach to equality, the smaller will be their mean square deviation and the more trustworthy will be our results. It may be urged, however, that the actual values of the mean square deviation, or *variance*, of Table VII are not directly comparable. Thus it is true that the variance of the results

TABLE VII
ACTUAL AND PERCENTAGE VARIANCE OF RESULTS

Station	1924	1925	1926	Mean
Charlottetown	46.54 260.22	155.13 949.95	83.31 341.65	91.40 517.27
Macdonald College	57.96 257.66	75.79 176.76	64.09 144.93	64.74 193.12
Scott	37.03 662.60	160.68 240.02	62.27 383.79	86.97 428.80
University of Alberta	326.33 318.99	209.28 191.22	139.03 204.86	219.30 238.02
Mean	109.79 374.87	156.60 389.49	87.82 268.81	117.18 344.39

obtained at the University of Alberta in 1924 is 326.33, whilst the corresponding figure for Scott is only 37.03. But it is also true that the mean yield of all plots at the University of Alberta in 1924 was 101.14 bushels per acre, whereas at Scott it was only 23.64 bushels per acre. The percentage variance is therefore shown below the actual variance in each case. This was obtained by multiplying the actual variance by $\left(\frac{100}{m}\right)^2$, where m is the mean yield of all plots for the particular station and season under consideration.

The individual percentage variance values exhibit considerable fluctuation. Considering the experiment as a whole, however, we see that there is little difference in the level of accuracy attained in the first two years; the mean variance in the third year being somewhat lower. The different stations fall into two groups. Scott and Charlottetown show a mean variance, over the three years of the experiment, of 428.80 and 517.27 respectively, whilst the corresponding figures for the University of Alberta and Macdonald College are 193.12 and 238.02.

The point, as to whether there exist any significant differences in precision, may be tested by performing an Analysis of Variance (5, p.190 *et seq.*) upon the 12 individual values. The sum of the squares of the deviations of the 12 values from their general mean is divided into three portions, as in Table VIII. The mean squares are obtained from the sums of squares by dividing by the appropriate number of degrees of freedom. Considering now these mean squares, it is apparent that the largest is that ascribable to differences in the average variance (over the three-year period) at the four stations. Fluctuation of the average percentage variance in the different years gives rise to the smallest mean square, interaction of place and year occupying an intermediate position. As an illustration of this interaction, in 1924 the percentage variance at Scott and Charlottetown respectively was 662.60 and 260.22. In 1925, however, the corresponding figures were 240.02 and 949.95.

TABLE VIII
ANALYSIS OF VARIANCE OF PERCENTAGE VARIANCE

	Degrees of freedom	Sum of squares	Mean square
Between years	2	34701.51	17350.75
Between places	3	213164.24	71054.74
Interaction of years and places	6	358603.02	59767.20
Total	11	606468.77	

The test, whether the mean square ascribable to one of these causes differs significantly from that ascribable to any other, is performed by calculating the quantity Z , equal to half the difference of the natural logarithms of the two mean squares, and consulting the table of Z (5, p.212) to discover if the observed value might reasonably be expected as a result of chance. In the present case, none of the values of Z obtainable are significant. We therefore conclude that, although the differences in percentage variance of yield between stations are considerable, yet taken as a whole the accuracy of the results obtained, both as between stations and between years, is sensibly of the same order.

TABLE IX
MEAN YIELD OF ALL PLOTS, IN BUSHELS PER ACRE

	1924	1925	1926	Mean
Charlottetown	42.29	40.41	49.38	44.03
Macdonald College	47.43	65.48	66.50	59.80
Scott	23.64	81.82	40.28	48.58
University of Alberta	101.14	104.61	82.38	96.04
Mean	53.62	73.08	59.64	62.11

The mean yield of all plots at the various stations during the three years, used in the calculation of the percentage variances, may be of some interest. The actual values are given in Table IX.

TABLE X
ANALYSIS OF VARIANCE OF MEAN YIELD

	Degrees of freedom	Sum of squares	Mean square
Between years	2	796.45	398.28
Between places	3	5001.94	1667.31
Interaction of years and places	6	1561.44	260.24
Total	11	7359.83	

The variance of these mean yields may be analyzed in precisely the manner adopted in the case of the percentage variance. Table X shows the results of this procedure.

The mean square attributable to differences between places is definitely larger than the interaction mean square. The value

of Z is 0.9287, whereas the probability of obtaining by chance a value equal to or greater than 0.7798 is only 0.05. It is therefore legitimate to conclude that there are significant differences in the general level of fertility at the four stations. The nature of these differences is apparent from Table IX. On the other hand, the differences in yield from year to year of the four stations taken as a whole are not significantly greater than those attributable to the interaction of place and year.

In endeavoring to estimate the regression of yield on seed rate, the field arrangement precludes our using the actual yields of plots sown at different rates, since these are obviously not directly comparable. We must instead utilize the mean differences in yield between the 16 plots of each seed rate and the adjacent 16 check plots grown at each station each year. In each case there will be four such differences, which may be regarded as values of a dependent variate. The differences in seed rate between the check and rate plots provide the corresponding values of the independent variate. Denoting the latter by x and the former by y , we calculate as our estimate of the coefficient of regression of y on x

$$b = \frac{S(x - \bar{x})(y - \bar{y})}{S(x - \bar{x})^2}$$

where \bar{x} and \bar{y} are the mean values of the two quantities respectively and S

indicates summation over all four individuals. The values of b thus obtained for the different varieties, places and years are set forth in Table XI.

To test whether any particular value of b differs significantly from zero, we calculate the quantities

$$s^2 = \frac{1}{n^1 - 2} S(y - Y)^2 \quad (n^1 = 4)$$

where y is the observed value and Y the corresponding value calculated from the regression equation, and

$$t = \frac{b\sqrt{S(x - \bar{x})^2}}{s}$$

The probability of obtaining by chance any observed value of t is then found from the table of "Student's" distribution. When this probability is 0.05 or less, the corresponding values of b are regarded as significant, and are marked with an asterisk in Table XI.

TABLE XI
AVERAGE INCREASE IN YIELD (BUSHEL PER ACRE) FOR EACH HALF-BUSHEL
INCREASE IN RATE OF SEEDING

		Abundance	Banner	Daubeney
Charlottetown	1924	0.685	0.795*	2.739*
	1925	0.286	3.181	3.571
	1926	1.001	0.498	2.371
Macdonald College	1924	0.075	0.490	-0.691*
	1925	...	1.083*	1.870
	1926	-0.419	0.214	1.979*
Scott	1924	-1.163*	-0.444	0.046
	1925	-0.211	-1.231	-0.048
	1926	-1.706	-0.542	0.682*
University of Alberta	1924	2.051	-2.946	1.634
	1925	-3.417	0.123	2.519
	1926	-0.092	0.643	1.720*

A study of Table XI indicates that out of the 35 regression coefficients obtained, only 8 may be regarded as definitely significant. In all the other cases the variations in yield observed were either so small, or else so irregular in nature that the resulting values of b could quite reasonably be supposed to have arisen purely through chance. Nevertheless, an examination of the figures does allow us to deduce certain conclusions which are not altogether devoid of interest.

It will be observed that of the eight significant coefficients, five are associated with the variety Daubeney, only two with Banner, and only one with Abundance. Furthermore, in the case of Daubeney these significant values are, with one exception, positive. It thus appears that the lower rates of seeding adopted for this variety have in fact been consistently below the optimum. The response of Daubeney to increases in the rate of seeding is, however, by no means the same at the different stations. The largest values of b are those

obtained at Charlottetown, where on the average the yield has been augmented by something more than 3 bushels per acre by each increase of $\frac{1}{2}$ bushel in the seed rate within the range studied. At Scott, on the other hand, the maximum value of b is 0.682, obtained in 1926, the values in 1924 and 1925 being quite insignificant. Macdonald College and the University of Alberta occupy a position intermediate between these two extremes. The differences in the value of b obtained from year to year at the same place are in general much less than the differences between places discussed above, though this statement must be modified in its application to Macdonald College. Here in 1924 the lower seed rates gave significantly better results, whereas in the remaining two years the position was reversed.

The results obtained with the other two varieties are subject to considerable fluctuation. On the whole, Banner shows a tendency towards increased yield with increased seed rate, and Abundance a tendency towards decreased yield with increased seed rate. As in the case of Daubeney, however, there are marked differences in the results at the four stations. Here again Scott and Charlottetown present the two extreme conditions. At Scott, both varieties gave negative values of b in all three years; at Charlottetown the values of b are all positive. At Macdonald College there would appear to be a definite tendency for Banner to give higher yields with increased seed-rate. The two years' results here available in the case of Abundance are quite inconclusive. At the University of Alberta, each of the three years has produced results differing both in magnitude and in nature from those of the other two. In 1924, the yield of Abundance was on the average increased by increasing the seed rate, whilst that of Banner was decreased. In 1925 and 1926 this situation was reversed. The observed differences in yield were in each case, however, of a very irregular nature. As a consequence, the standard errors of the regression coefficients are in all cases so large as to destroy their significance.

Instead of calculating a separate regression coefficient for each year, we may estimate the average regression of yield on rate of seeding for all three years. This has been done, and the resulting values of b are shown in Table XII.

TABLE XII
AVERAGE INCREASE IN YIELD (BUSHELS PER ACRE)
FOR EACH HALF-BUSHEL INCREASE IN SEED RATE.
AVERAGE OF THREE YEARS' RESULTS

	Abundance	Banner	Daubeney
Charlottetown	0.657	1.491	2.894*
Macdonald College	-0.172	0.595*	1.052
Scott	-1.004	-0.739	0.226
University of Alberta	-0.486	-0.728	1.958*

When the results of the three years were averaged in this way, it was found that in the case of the variety Abundance at Scott Experimental Station, and Daubeney at Macdonald College, the yield showed a definite tendency to increase with increased seed rate up to a certain

point only. After this, any further increases in rate of seeding not only produced no further increase in yield, but actually exercised a depressive effect. It is therefore not surprising that no significant value of the regression co-

efficient was obtained when a straight line was fitted to the data. When a parabolic curve of the form

$$(y - \bar{y}) = b(x - \bar{x}) + c(x^2 - \bar{x}^2)$$

was fitted, however, significant values were obtained for the parameter c . The actual values were:

Scott, Abundance	$b = 0.149$,	$c = -0.670^*$
Macdonald College, Daubeney	$b = 1.052$,	$c = -1.009^*$

Tendencies of a similar nature were also to be perceived in the results of certain other varieties and stations, most notably Banner at Macdonald College; but in no case could the value of the quadratic coefficient c obtained be regarded as significant.

Of the twelve combinations of variety and station, therefore, five have given significant results when the three-year averages are considered. The nature of these results is indicated in Fig. 3. The average yields of all the check plots of any particular variety grown at any particular station have been taken as starting points; then, using the coefficients of Table XII, except in the two cases where a significant quadratic term was obtained, the curves of regression have been constructed.

The first point to be noted in a consideration of the results portrayed in Fig. 3 is that when all the varieties are sown at rates in the vicinity of their respective optima, the order of yield at Charlottetown, Macdonald College, and the University of Alberta is: Banner, Daubeney, Abundance; though at the two latter stations the differences between Daubeney and Abundance cannot be regarded as significant. At Scott however the order is: Banner, Abundance, Daubeney. The second point is that at Macdonald College, the University of Alberta, and Charlottetown, the relative positions of Daubeney and Abundance could be reversed by a suitable choice of seed rates. At Scott, the differences are not so striking, but their effect upon the comparison of Abundance with the other two varieties is by no means negligible.

Banner has consistently outyielded the other two varieties, at all stations and at all rates of seeding. Its superiority is more marked at the University of Alberta than at any of the other stations. In fact, it would appear that had higher rates of seeding Daubeney been employed at Charlottetown the difference in yield between this variety and Banner might have been reduced to quite insignificant proportions.

We may therefore conclude, from our examination of the data thus far, that variations in the rate of seeding have perceptibly influenced the relative yielding capacities of the three experimental varieties at all four stations; also, that even when the optimum seed rates are employed in each case, the relative performance of the three varieties is by no means the same at all stations. Whilst there can be little doubt that the observed variations are indicative of a real effect, it is nevertheless necessary to keep in mind the considerations developed in Section 4 of this paper. Significant differences in yield between plots sown at different rates have indeed been demonstrated; but we cannot disregard entirely the possibility that a sensible portion of this variation may in fact be attributable to differences in the fertility of the various plots.

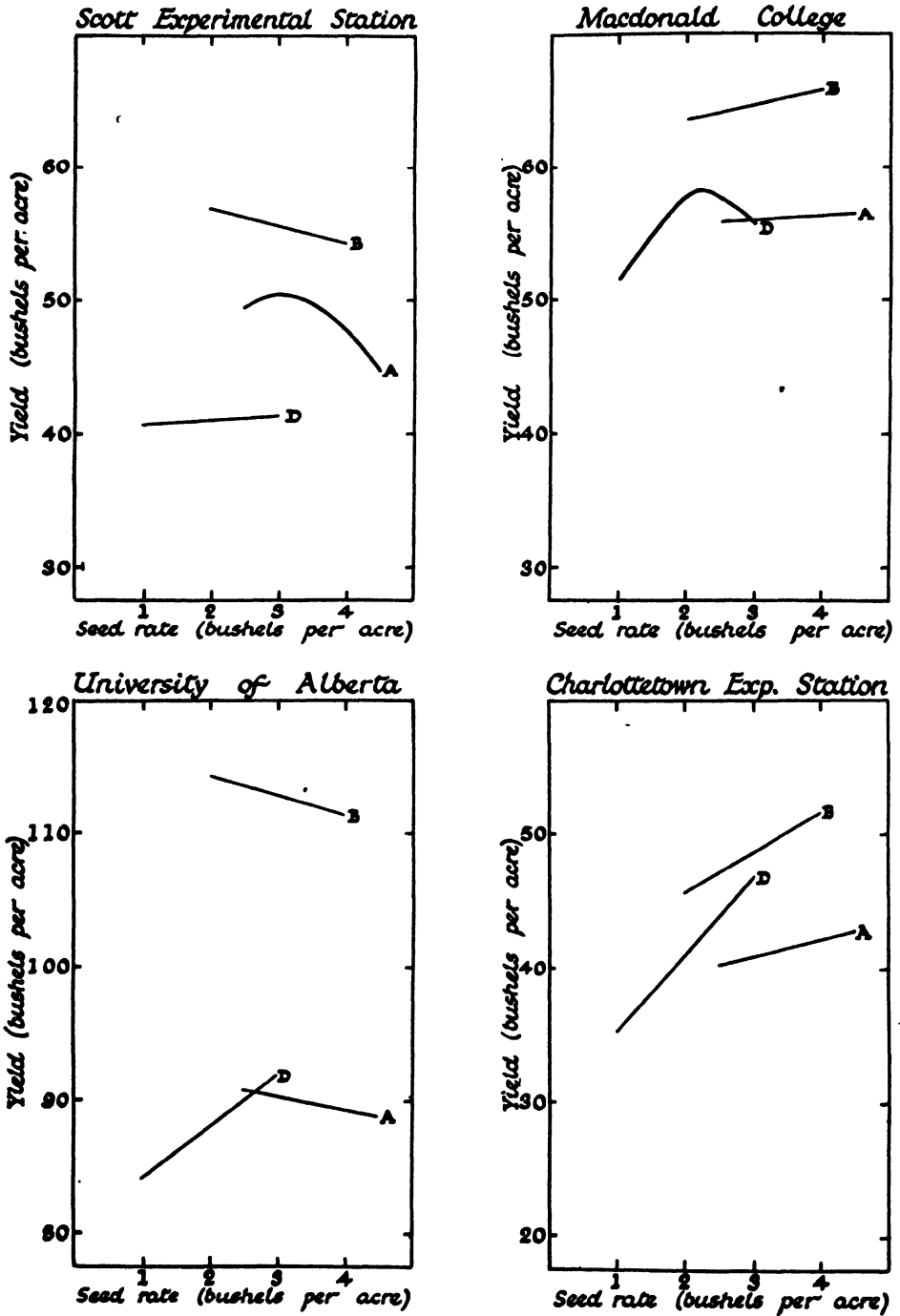


FIG. 3. Curves of regression of yield on rate of seeding. Average of three years' results. A = Abundance, B = Banner, D = Daubeney.

Having demonstrated differences in the behavior of these varieties as regards yield, we may next proceed to enquire to what extent these differences are to be associated with differences in such characteristics as size of seed and tillering capacity. Obviously the first step in any such enquiry is to ascertain the nature and magnitude of the differences which are to be observed between the three varieties in these respects.

TABLE XIII
WEIGHT PER 1000 KERNELS, IN GRAMS, OF THE SEED OF THE THREE VARIETIES USED

Year	Station	Abundance	Banner	Daubeney
1924	Charlottetown	39.7	34.4	21.2
	Macdonald College	39.0	34.2	22.8
	Scott	39.0	34.2	22.8
	Univ. of Alberta	39.0	34.2	22.8
1925	Charlottetown	32.2	30.1	20.7
	Macdonald College	...	29.3	23.0
	Scott	36.4	30.8	21.9
	Univ. of Alberta	28.7	29.1	21.1
1926	Charlottetown	33.6	33.6	25.2
	Macdonald College	33.6	33.6	25.2
	Scott	33.6	33.6	25.2
	Univ. of Alberta	33.5	35.5	25.5

Table XIII shows the estimates of the weight per 1000 kernels of the seed of the different varieties used at each station in the years 1924, 1925 and 1926. The agreement between the figures supplied by the Dominion Cerealists and those obtained by the different stations was on the whole good, except in the case of the variety Abundance in 1925. In this case, discordant results, ranging from 28.7 gm. at the University of Alberta to 36.4 gm. at Scott Experimental Station, were obtained. Apart from this instance, however, there is no reason to suppose that the various lots of seed used in any one year were other than homogeneous.

When the differences between varieties are examined, it appears that in each year the seed of Daubeney had a considerably lower weight per 1000 kernels than that of either Abundance or Banner. The differences in this respect between the two latter varieties are however in all cases small, and, except in 1924, quite insignificant. Considering the yield figures in the light of these observations, we might be led to conclude that the optimum concentration of plants is greater in the case of Daubeney than in the case of the other two varieties, at all stations except Macdonald College. The matter is much complicated, however, by the fact that the stand of plants actually obtained bears by no means a constant relation to the number of seeds planted. A study of the original data shows that considerable fluctuation in the observed stand of plants occurred not only from year to year and from place to place, but also amongst adjacent plots.

There is also, unfortunately, good reason to suspect that an appreciable proportion of this fluctuation is due to certain errors inseparable from the estimation of the actual stand.

The method of estimation adopted has already been described. At harvest time, the centre row of each nine-row plot was dug up in its entirety and the number of individual plants and the total number of tillers (culms) counted. The counting of the total number of culms is of course quite straightforward; but that of the individual plants is often a matter of very considerable difficulty. Groups of plants are often found crowded very closely together, their root systems inextricably intermingled; and any attempt to disentangle them may, and undoubtedly often does, lead either to incomplete separation, on the one hand, or to the pulling apart of tillers, on the other. In the first case two or more individuals are in fact only counted as one, whilst in the second, one actual individual may give rise to a "count" of two or three.

It may be suggested that these two opposing errors are likely to occur with approximately equal frequency in the counting of a given plot, but this does not appear to be the case. The difficulty is most acute where the plants grow most luxuriantly, as on the highly fertile soil at the University of Alberta. Here in 1924 no fewer than 39 counts of plants in excess of the number of germinable seeds sown were recorded; the "percentage stand" being in one case 149. On the other hand, it is by no means uncommon to find that, in the case of two adjacent plots the percentage stand of one is estimated at 110 or so and that of its neighbor at only 60 or 70.

It is of course obvious that these errors affect the estimates of both percentage stand and degree of tillering, though in an opposite sense. Thus, the adherence of two or more plants results in an underestimate of the stand of plants and an overestimate of the degree of tillering; whilst the breaking apart of tillers belonging to the same plant causes an overestimate of stand and an underestimate of tillering.

In addition to errors of this kind affecting the actual operation of counting, another and quite distinct source of inaccuracy in the results arises out of the fact that the centre row of each plot may be by no means representative of the plot as a whole. We have therefore *errors of sampling* as well as errors in the procedure applied to the sample. These would of course persist no matter how precisely the actual counting was performed, and since only one sample was taken from each plot, their magnitude is incapable of determination.

Although the estimate of stand obtained from any individual plot is therefore of doubtful value, yet when the numerous such estimates made in the case of each variety at each station each year are considered in the aggregate, certain definite tendencies reveal themselves. This is illustrated in Table XIV, where the average percentage stand of the different varieties at each station during the three years is to be found.

The values in this table are the average of 64 actual determinations, except in some cases in which the total number of plots was not grown or for other reasons counts were not available. They may therefore be considered to give rather more than a mere indication of the general order of the stand of plants actually obtained; for the combination of a large number of observations, though each is subject to such errors as to render them individually of little value, produces an estimate of considerably greater precision. The results

obtained at Scott have been included in the table, but in making comparisons it must be remembered that in 1925 the counts at this station were made immediately after the emergence of the plants in the spring.

TABLE XIV
AVERAGE STAND OF PLANTS, EXPRESSED AS A PERCENTAGE OF THE NUMBER OF
GERMINABLE SEEDS SOWN

Year	Station	Abundance	Banner	Daubeney
1924	Charlottetown	81.3	89.0	85.3
	Macdonald College	54.1	52.1	55.0
	Scott	88.2	84.2	87.3
	Univ. of Alberta	86.9	95.3	88.1
1925	Charlottetown	81.1	71.2	68.2
	Macdonald College	61.4	73.3
	Scott	91.1	94.0	93.2
	Univ. of Alberta	81.7	85.5	83.2
1926	Charlottetown	76.2	75.7	71.8
	Macdonald College	57.5	58.8	58.2
	Scott	74.5	70.1	66.8
	Univ. of Alberta	87.6	86.1	79.6

The most obvious feature of Table XIV is the uniformly low stands of plants obtained at Macdonald College, and the uniformly high ones obtained at the University of Alberta. The effect of the spring count at Scott in 1925 is also very apparent, although it is of course impossible to assert that the large values observed are due entirely to that cause. Counts of the number of plants which emerged in the spring and those surviving to maturity, carried out at all four stations, might have produced results of some interest.

The differences to be observed between varieties, or between the results of the three years, are by no means so distinct. Their precise nature may best be arrived at by an analysis of the variance of the percentage stand figures. Unfortunately, when this is undertaken it is necessary to discard the whole of the results obtained at Scott. A further difficulty arises from the fact that the variety Abundance was not grown at Macdonald College in 1925. In this case however it is possible to estimate a "most likely value" of the missing quantity, using the method developed by Allen and Wishart (1) for the estimation of the yield of a missing plot in field experiments.

This value is estimated from the results obtained at the other stations, the comparative performance of Abundance at Macdonald College in the two other years, and the observed stand of the two other varieties at that station in the year in question. This estimated value is not designed to supply any new information, but simply to fill in the gap and render the data amenable to the processes of the Analysis of Variance, so that the information contained in the observations which actually were made may be extracted. This is the only justification for its employment in the present connection. The present instance well illustrates the undesirable effect which can be produced in experiments of this nature by deviations from uniformity of procedure.

TABLE XV
ANALYSIS OF VARIANCE OF AVERAGE PERCENTAGE STAND

Variance due to	Degrees of freedom	Sum of squares	Mean square	Z
Years	2	76.28	38.14	2.1002*
Places	2	3,625.64	1812.82	
Varieties	2	12.93	6.47	
Interaction variety and place	4	135.46	33.87	0.6330
Interaction variety and year	4	59.62	14.90	
Interaction place and year	4	385.51	96.38	
Remainder	7	190.23	27.18	
Total	25	4,485.66		

The analysis of variance takes the form shown in Table XV. Of the various "mean squares" obtained only two are appreciably larger than the "remainder". They are the ones attributable to general differences between stations and to the interactions of place and year respectively. The values of Z appropriate to these are given in the last column of the table. The first of these is such as would be expected by chance less than once in 100 trials; the second, however, cannot be regarded as significant. We therefore conclude that the only systematic differences of any significance are those between the average results, over the whole three years, obtained at the various stations.

The foregoing analysis deals only with the average stand of all the plots of any one variety grown at each station each year. It is however obviously a matter of some interest to inquire into the possibility of differences in percentage stand between plots of the same variety sown at different rates. This may be attempted by utilizing the averages of the counts of plants made on the centre rows of the eight replicate plots of each seed rate, and the corresponding averages derived from the eight adjacent check plots. Knowing the actual number of germinable seeds sown, and the actual number of plants counted, a series of 2 by 2 tables of the following form may be constructed.

	Produced plants	Failed to produce plants	Total seeds sown
Check	160	143	303
Rate	111	91	202
Total	271	234	505

If the percentage stand was in fact the same in both sets of plots we should expect the quantities 160, 143, and 111, 91, to be in proportion. The question as to whether there is a significant difference in stand therefore resolves it-

self into the question as to whether the observed frequencies differ significantly from the "proportional" values calculated from the marginal totals. The usual procedure in such cases is to calculate the quantity $\chi^2 = S \left(\frac{(x-m)^2}{m} \right)$, where x is the number observed, m the number expected, in this case on the assumption

that the percentage stand is the same in both check and rate plots, and S denotes summation over the four "cells" of the table. In this instance, however, we shall find it advantageous to use the quantity $\sqrt{\chi^2}$, with the convention that when the proportion of plants is higher in the check plots than in the rate plots, $\sqrt{\chi^2}$ shall be taken as positive, and when the proportion of plants is higher in the rate plots than in the check plots, as negative.

It has been shown (3) that when the two sets of observations are in fact random samples from a homogeneous normal population, the quantity $\sqrt{\chi^2}$ will be normally distributed about zero with unit standard deviation. Consequently, if a value of $\sqrt{\chi^2}$ exceeding 2 in absolute magnitude is obtained, there is good reason to believe that the observed proportion of plants maturing, or percentage stand, is not the same in the two sets of plots.

The actual values of $\sqrt{\chi^2}$ obtained in this way are set forth in Table XVI. There are two gaps in the table. The first of these is due to the variety Abundance not being grown at Macdonald College in 1925. The second is due to the fact that at the University of Alberta in 1924 the average number of plants counted in the 2 and $2\frac{1}{2}$ bushel plots of the variety Banner was greater than the number of germinable seeds sown. The pulling apart of tillers of the same plant during the counting process, and errors in seeding may both have contributed in some measure to this result.

Of the 138 values of $\sqrt{\chi^2}$, no fewer than 34 exceed 2.000 in magnitude. There is thus no doubt of the significance of the differences in percentage stand *recorded* in the case of adjacent check and rate plots. It is necessary to bear in mind however that for reasons already made clear, the numbers of plants recorded may differ to a greater or less extent from the numbers actually growing in the various plots; and the results of Table XVI must therefore be interpreted with some reserve. Nevertheless, it does appear that the percentage stand in the check and rate plots cannot be regarded as uniform.

There is a general tendency (to which numerous exceptions are to be noted) for negative values of $\sqrt{\chi^2}$ to be associated with comparisons in which the rate plot is seeded at a lower rate than the check, and positive values to be associated with those in which the situation is reversed. This is quite understandable. The counts being made in the fall, might indeed be expected to exhibit some evidence of the more intense competition prevailing in the more heavily seeded plots.

It will be observed that the varieties Abundance and Daubeney at Scott Experimental Station in 1925 are amongst the exceptions to this tendency. This also was to be expected, since the effects of plant competition would hardly be apparent at the time (spring) when these counts were made. In the case of Banner, the lower rates of seeding do actually give negative values and the higher rates of seeding positive ones, though none of these are significant.

The largest values of $\sqrt{\chi^2}$, and hence the greatest differences in percentage stand, are to be found associated with the variety Daubeney. Of the four stations, the University of Alberta and Macdonald College exhibit the most pronounced signs of variability in this respect.

TABLE XVI
SIGNIFICANCE OF DIFFERENCES IN PERCENTAGE STAND BETWEEN PLOTS SOWN AT DIFFERENT SEED RATES. VALUES OF $\sqrt{\chi^2}$

	Abundance				Banner				Daubeney			
	2½ bu.	3½ bu.	4 bu.	4½ bu.	2 bu.	2½ bu.	3½ bu.	4 bu.	1 bu.	1½ bu.	2½ bu.	3 bu.
Charlottetown 1924	0.712	1.385	2.719	1.430	0.173	-0.281	0.757	-0.302	-0.704	-1.158	0.647	1.996
1925	0.990	0.544	0.126	2.793	-0.100	1.308	1.401	2.004	-0.327	2.983	1.322	1.998
1926	2.201	-0.574	1.361	3.128	-0.122	1.382	-2.364	1.778	2.158	-0.156	1.624	2.141
Macdonald 1924	-0.697	-1.767	-0.444	-2.011	-0.593	1.445	0.977	0.308	-2.166	-1.707	-1.313	-2.801
1925	-3.347	-0.690	0.616	2.830	-3.557	-0.907	2.623	6.306
College 1926	-2.153	-0.978	0.407	2.684	-0.473	1.129	6.259	3.847	-2.107	-1.133	0.491	1.543
Scott 1924	-1.460	-1.828	0.632	0.715*	0.158	-0.349	0.266	0.362	0.658	-1.157	1.712	2.658
1925	-0.152	2.242	0.489	0.653	-0.430	-0.550	0.656	0.729	0.742	0.836	0.502	-2.469
1926	-0.409	-0.438	-0.701	0.888	-1.185	-0.937	1.045	-0.849	0.690	-1.290	-2.484	-1.567
University 1924	-0.628	1.169	0.000	1.138	3.766	3.582	-4.042	0.220	5.127	7.710
of Alberta 1925	0.071	0.171	-0.076	0.960	0.264	0.000	0.516	0.182	-0.902	-0.455	-0.583	0.818
1926	-2.890	-0.941	1.299	2.326	-0.838	-0.686	3.366	3.701	-1.456	-0.264	2.415	0.443

* In 1924, 4½ bu.

TABLE XVII

THE INCIDENCE OF TILLERING. AVERAGE NUMBER OF TILLERS PER PLANT

Year	Station	Abundance	Banner	Daubeney
1924	Charlottetown	1.04	1.03	1.33
	Macdonald College	1.29	1.19	1.45
	Scott	1.01	1.03	1.07
	Univ. of Alberta	1.16	1.01	2.37
1925	Charlottetown	1.16	1.23	1.53
	Macdonald College	1.01	1.28	1.69
	Univ. of Alberta	1.01	1.03	1.49
1926	Charlottetown	1.10	1.04	1.70
	Macdonald College	1.40	1.46	1.86
	Scott	1.24	1.21	1.36
	Univ. of Alberta	1.00	1.02	1.58

Differences in the degree of tillering of the three varieties may be examined in precisely the same way as were differences in the average percentage stand. Table XVII gives the average number of tillers per plant appropriate to the different varieties, stations and years. Table XVIII shows the results of an analysis of the variance of the values obtained at Charlottetown Experimental Station, Macdonald College, and the University of Alberta.

TABLE XVIII

ANALYSIS OF VARIANCE OF AVERAGE NUMBER OF TILLERS PER PLANT

	Degrees of freedom	Sum of squares	Mean square	Z
Years	2	0.0107	0.0054	0.3783 1.4653*
Places	2	0.1845	0.0922	
Varieties	2	1.5829	0.7915	
Interaction variety and place	4	0.2119	0.0530	
Interaction variety and year	4	0.0515	0.0129	
Interaction place and year	4	0.3581	0.0895	
Remainder	7	0.2957	0.0422	
Total	25	2.6953		

It is apparent from Table XVII that the variety Daubeney has consistently produced more tillers per plant than either Abundance or Banner. Except at Macdonald College, and at Scott in 1926, the tillering of the two latter is in fact very slight.

This varietal effect is the only one to appear significant in the analysis of variance. It is moreover possible to carry the analysis one stage further than that indicated in Table XVIII. The sum of squares appropriate to varietal differences may be subdivided into two portions, one due to the differences between Daubeney and the mean of the other two varieties, and the other due to differences between the two latter. When this is done, it is found that of the sum of squares, 1.5829, no less than 1.5811 is attributable to the differences

between Daubeney and the mean of Abundance and Banner, and only 0.0018 to differences between these two. Thus the only significant feature of the tillering figures is that Daubeney has consistently tillered more freely than the other two varieties, the differences in performance between Abundance and Banner being quite negligible.

It might be thought desirable to attempt an examination into the effect of thickness of stand on tillering in the case of the different varieties; and precise information on this point would indeed have been of some interest. Upon a consideration of the nature of the errors to which the observations are subject, however, the present data do not appear suitable for this purpose. It has already been pointed out that any underestimate of the stand of plants is automatically accompanied by an overestimate of the amount of tillering, and *vice versa*. In this way a certain spurious correlation is introduced into the observed results. Since there is reason to believe that errors of this kind of some magnitude have in fact occurred, conclusions of any exactitude would seem to be rendered impossible.

We may, however, construct a table, such as Table XIX, showing the average number of tillers per plant observed in the plots devoted to the different seed rates. Of course the stands of plants in the individual replicate plots may vary considerably amongst themselves, but the average stand of plants in, for example, the plots sown at the rate of 4 bushels per acre will in general be distinctly denser than that in the plots of the same variety sown at the rate of $3\frac{1}{2}$ bushels per acre.

The effect of increased density of plants in reducing the amount of tillering is apparent at all stations except Macdonald College, where the results are so conspicuously irregular as to be in a class by themselves. There is little to choose between the results obtained with Abundance and Banner; and certainly nothing to suggest that the former variety is in any way inherently inferior in tillering capacity. The variety Daubeney on the other hand shows not only a higher average number of tillers per plant, as appears from the preceding analysis of variance, but also a much greater flexibility in tillering at the different seed rates. This is particularly marked at the University of Alberta, where the high fertility of the soil doubtless facilitated increased tillering with thinner seeding.

Besides the evidently greater inherent tillering capacity of Daubeney, two other circumstances may have contributed in some degree to its greater range of tillering. In the first place, the seed of this variety having a much lower weight per 1000 kernels than that of Abundance and Banner, the range from the highest to the lowest number of seeds sown will be correspondingly greater. So, presumably, will the range of density of the plant population, especially in the earlier part of the growing season, when tillering would be taking place. By the fall, when the plant counts are made, the different intensities of plant competition may be expected to have modified this situation somewhat; and it will be remembered that the largest values of $\sqrt{\bar{x}}$ in Table XVI are in fact associated with the variety Daubeney. The second point is that the lowest

TABLE XIX
AVERAGE NUMBER OF TILLERS PER PLANT AT VARIOUS SEED RATES

	Abundance						Banner				Daubeney				
	2½ bu.	3 bu.	3½ bu.	4 bu.	4½ bu.	2 bu.	2½ bu.	3 bu.	3½ bu.	4 bu.	1 bu.	1½ bu.	2 bu.	2½ bu.	3 bu.
Charlottetown	1.06	1.04	1.04	1.02	1.02	1.04	1.04	1.03	1.02	1.02	1.54	1.49	1.32	1.18	1.20
	1.25	1.15	1.15	1.13	1.24	1.22	1.31	1.22	1.24	1.21	1.56	1.52	1.51	1.50	1.58
	1.63	1.02	1.02	1.02	1.02	1.04	1.01	1.05	1.01	1.02	2.61	1.83	1.77	1.60	1.46
Macdonald College	1.34	1.27	1.14	1.17	1.10	1.34	1.13	1.17	1.14	1.22	1.94	1.30	1.39	1.23	1.27
	1.13	1.34	1.31	1.33	1.57	1.88	1.91	1.63	1.54	1.82
	1.38	1.41	1.32	1.42	1.51	1.78	1.46	1.26	1.59	1.67	1.90	2.43	1.75	1.69	1.71
Scott	1.01	1.01	1.00	1.00	1.00	1.12	1.03	1.01	1.00	1.00	1.39	1.05	1.02	1.01	1.01

	1.15	1.25	1.18	1.17	1.37	1.23	1.27	1.23	1.17	1.11	2.09	1.38	1.31	1.09	1.11
University of Alberta	1.30	1.18	1.13	1.09	1.06	1.04	1.02	1.01	1.01	1.00	3.74	2.79	2.14	2.02	1.89
	1.04	1.01	1.00	1.00	1.01	1.16	1.06	1.01	1.00	1.00	2.36	1.70	1.40	1.16	1.14
	1.01	1.01	1.00	1.00	1.00	1.06	1.04	1.01	1.02	1.00	2.48	1.95	1.48	1.20	1.10

seed rate of Daubeney is considerably below that of the other two varieties, the conditions for tillering thus being made more favorable.

We may next consider to what extent the observed differences in weight per 1000 kernels of seed and in tillering capacity, and the fluctuations in percentage stand have affected the yield of the three varieties.

Reference to Tables XI and XII at once reveals that the variety Daubeney, which has shown the greatest tillering capacity and might therefore be expected to adjust itself more readily to different rates of seeding, has on the contrary shown the most pronounced variation in yield. This situation may perhaps be to some extent accounted for by the considerations advanced in discussing the tillering results. The smaller weight per 1000 kernels of this variety results in a much wider range of plant densities, whilst the lower rates of seeding are definitely below that optimum range within which variations in the seed rate appear to have very little effect on yield. The greater degree of tillering in evidence at the lower seed rates is apparently not sufficient to offset the reduction in yield caused by the diminution of the plant population.

The effect of differences in percentage stand would seem to require some investigation, in view of the considerable fluctuation in this respect observed from plot to plot. The obvious method to adopt would appear to be to determine the regression of yield on plant number, as opposed to seed rate.

There are several ways in which this may be done.

The first to be adopted was that of obtaining separate regression coefficients for each variety, place, and year, as was done in the case of rate of seeding with the results shown in Table XI. In the present instance, however, the values of the regression coefficient were not determined from 4 yield-figures, each the average of 16 differences between adjacent check and rate plots, but from 32 yield-figures, the exact nature of which may best be understood by reference to the accompanying diagram (Fig. 4).

Here R_1 , R_2 , and C_1 , C_2 , represent yields of individual three-row check and rate plots, n_1 , n_2 , and n_3

the plants counted in the central rows of the nine-row plots, of which there were eight for each rate of seeding. Then the difference in plant number used in calculation is taken as $n_2 - \frac{n_1 + n_3}{2}$ and the difference in yield as

$R_1 + R_2 - C_1 - C_2$, precisely the quantity used in calculating the values of t at the beginning of the analysis. The total number of these differences which may be formed within each variety is 32.

The values of the regression coefficients obtained are given in Table XX. These represent the average increase in yield for each increase in stand, over the stand of the check plots, of 100,000 plants per acre.

An examination of Tables XI and XX reveals that although the actual magnitudes of the various regression coefficients are of course different, their relative order is much the same in the two cases. There are however certain points of dissimilarity worth noting.

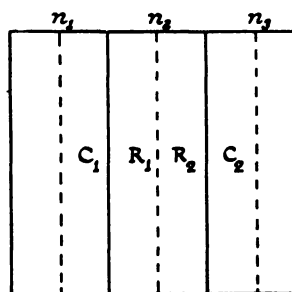


FIG. 4

TABLE XX

AVERAGE INCREASE IN YIELD (BUSHELS PER ACRE), FOR EACH INCREASE OF 100,000
IN THE NUMBER OF PLANTS PER ACRE

		Abundance	Banner	Daubeney
Charlottetown	1924	0.420	0.232	0.884*
	1925	-0.089	2.078*	1.414*
	1926	0.366	0.194	0.880*
Macdonald College	1924	0.005	0.330	-0.330
	1925	0.005	0.391	1.532*
	1926	0.374	0.233	1.268*
Scott	1924	-0.810*	-0.219	0.026
	1925	-0.110	-0.471	-0.017
	1926	-0.924*	-0.381	0.330
University of Alberta	1924	1.688	-0.114	0.809
	1925	-1.301*	0.094	0.978*
	1926	-0.729	-0.412	0.750*

The total number of coefficients which may be regarded as statistically significant is now 11, as compared with 8 in Table XI. These are indicated in Table XX by an asterisk. Of the 11, 7 are found to belong to the variety Daubeney, which has at all stations excepting Scott shown a definite tendency towards higher yield with increased stand of plants. Furthermore the depression in yield with increasing seed rate, noted in the case of this variety at Macdonald College in 1924, though still in evidence, loses its significance when related to plant number.

Considering now the other two varieties, Abundance has shown a definite tendency to give lower yields from the denser stands of plants in two out of the three years at Scott. At the University of Alberta the results are more irregular, but a significant negative value of the regression coefficient is obtained for 1925. At Macdonald College and Charlottetown no significant tendency in either direction is discernible. The only significant result in the case of Banner is that at Charlottetown in 1925. It may however be more than a coincidence that at Scott negative values of the regression coefficient, and at Macdonald College positive values, were obtained in all three years.

An attempt was next made to develop for each of the 35 combinations of variety, station, and year a quadratic regression equation, such as was obtained in two instances when considering the relation between yield and rate of seeding. The values of the quadratic coefficients resulting were however uniformly insignificant.

This was somewhat disappointing, as it had been hoped by this method to determine the optimum stand of plants of each variety at the different stations. It was realized that the result was probably to a considerable extent attributable to errors in the counts of plants. Nevertheless it was thought desirable to investigate to what extent the observed results might be influenced by variations in the general fertility level of the land on which plots sown at

TABLE XXI
MEAN YIELD IN BUSHELS PER ACRE OF CHECK PLOTS ADJACENT TO PLOTS SOWN AT VARIOUS RATES

	Abundance Rate plots sown at				Banner Rate plots sown at				Daubeney Rate plots sown at				
	2½ bu.	3½ bu.	4 bu.	4½ bu.	2 bu.	2½ bu.	3½ bu.	4 bu.	1 bu.	1½ bu.	2½ bu.	3 bu.	
Charlottetown	1924	40.65	33.03	31.95	46.55	47.51	53.33	50.43	64.55	33.08	30.96	33.06	40.84
	1925	35.91	44.58	52.54	45.26	42.80	43.22	45.25	42.20	33.20	33.13	34.63	38.16
	1926	26.04	39.18	43.91	40.83	44.56	47.33	56.90	62.04	64.95	51.57	57.57	61.25
Macdonald College	1924	52.34	43.71	41.62	52.02	49.52	54.98	52.58	51.81	42.27	37.22	36.46	50.93
	1925	60.90	72.97	70.86	72.51	58.78	62.91	68.00	64.82
	1926	58.68	66.53	59.19	64.53	72.85	74.08	74.70	71.61	47.52	75.03	68.27	64.49
Scott	1924	25.77	28.48	24.93	17.43	37.11	32.67	25.07	21.87	32.38	15.10	15.86	12.35
	1925	82.84	87.28	83.25	90.20	88.10	88.98	86.90	91.44	72.04	72.65	72.30	70.83
	1926	38.75	36.97	33.84	35.01	54.60	56.68	47.24	45.33	35.85	34.55	36.33	31.27
University of Alberta	1924	92.10	84.82	90.03	112.04	124.09	124.71	132.96	124.35	87.71	84.56	78.82	86.52
	1925	94.22	89.32	91.58	97.98	123.55	126.60	113.95	114.15	101.67	106.15	99.97	106.50
	1926	89.02	78.14	89.17	89.51	84.38	91.30	88.48	102.68	68.67	73.88	67.64	69.12

different rates were grown. The nature of these variations has been discussed in Section 4, where it was also pointed out that the field arrangement made it possible to demonstrate their existence only in the case of different sets of plots of the same variety.

For this purpose the "range" of plots of each variety grown at any particular station may be subdivided into four portions. These four portions, which consist of all the plots of any one seed-rate and their adjacent check plots (32 three-row plots in all), we may term "blocks". It is with the check plots, of which there are 16 in each block, that we are concerned, for these being sown at a uniform rate, their yields allow us to judge whether the general level of fertility of the various blocks may be regarded as the same or not.

In Table XXI will be found the average yield of the 16 check plots in the blocks containing the replicate plots of the various seed rates. It will be observed that the mean yield varies from block to block, often by a considerable amount. To test the significance of these variations is to test whether the variation between blocks of plots is significantly greater than the variation between plots within the same block.

This resolves itself into a simple analysis of the variance of the yields of the 64 check plots in each range. The sum of the squares of the deviations of the 64 observed yields from their general mean may be divided into a portion representing the deviations of the four block means from the general mean, and a portion representing the deviation of the 16 plot yields in each block from the mean yield of their particular block. When these quantities are divided by the appropriate number of degrees of freedom (namely 3 and 60), mean square deviations, or "variances", "within blocks" and "between blocks" are obtained. The Z test may be used to determine whether the variance between blocks is significantly greater than that within blocks.

Table XXII gives the sums of squares, mean squares, and values of Z obtained by applying this process to the yield figures of each station. The number of degrees of freedom available in each case has also been given, since the full complement of 64 yields was not always available. The last column of Table XXII gives the 1% value of Z , *i.e.*, the value which is expected to be equalled or exceeded by chance only once in 100 times.

It will be observed that in certain instances (for example, Banner and Daubeney at Scott in 1925) no value of Z is given. In these cases, which are few in number, the variance "within blocks" is actually greater than that "between blocks". In the great majority of cases, however, the reverse condition holds, and some of the values of Z obtained are very large indeed.

The differing effects of soil heterogeneity, encountered at the various stations, as revealed by these figures, are of some interest. Thus at Scott in 1924 the arrangement of the plots appears to have coincided to a marked extent with systematic variations in soil fertility (see Table XXI); and values of Z in the neighborhood of 2.000 are obtained, whereas the 1% value is only 0.7100. In 1925 however the variation between blocks is of a very much lower order. Two of the varieties indeed gave a variance "within blocks" greater than that "between blocks". In 1926 the variance between blocks was more

TABLE XXII
ANALYSIS OF VARIANCE OF CHECK PLOT YIELDS

Variety	Variance	Degrees of freedom	Sum of squares	Mean square	Z	1% Z
Charlottetown 1924 Abundance	Between blocks	3	2190.23	730.08		
	Within "	59	2560.02	43.39	1.4124	0.7100
Banner	Between blocks	3	2546.77	848.92		
	Within "	59	5177.08	87.75	1.1462	0.7100
Daubeney	Between blocks	3	867.88	289.29		
	Within "	59	1236.08	20.95	1.3127	0.7100
Charlottetown 1925 Abundance	Between blocks	3	2147.66	715.88		
	Within "	58	2327.95	40.14	1.4406	0.7114
Banner	Between blocks	3	83.77	27.92		
	Within "	60	5951.47	99.19
Daubeney	Between blocks	3	266.09	88.70		
	Within "	60	2609.63	43.49	0.3564	0.7086
Charlottetown 1926 Abundance	Between blocks	3	2980.17	993.39		
	Within "	60	2888.35	48.14	1.5136	0.7086
Banner	Between blocks	3	3200.90	1066.97		
	Within "	60	4214.04	70.23	1.3604	0.7086
Daubeney	Between blocks	3	1561.28	520.42		
	Within "	60	3802.13	63.37	1.0028	0.7086
Macdonald College 1924 Abundance	Between blocks	3	1483.22	494.41		
	Within "	60	2290.55	38.18	1.2806	0.7086
Banner	Between blocks	3	235.37	78.46		
	Within "	60	3190.69	53.18	0.1944	0.7086
Daubeney	Between blocks	3	2130.22	710.07		
	Within "	60	2638.39	43.97	1.3910	0.7086
Macdonald College 1925 Banner	Between blocks	3	1549.01	516.34		
	Within "	60	2875.18	47.92	1.1886	0.7086
Daubeney	Between blocks	3	712.36	237.45		
	Within "	60	2408.86	40.15	0.8881	0.7086
Macdonald College 1926 Abundance	Between blocks	3	660.79	220.26		
	Within "	55	2381.82	43.31	0.8133	0.7160
Banner	Between blocks	3	87.35	29.12		
	Within "	50	1103.62	22.07	0.1386	0.7234
Daubeney	Between blocks	3	5426.90	1809.00		
	Within "	60	6531.06	108.85	1.4053	0.7086

TABLE XXII—Continued

Variety	Variance	Degrees of freedom	Sum of squares	Mean square	Z .	1% Z
Scott 1924 Abundance	Between blocks	3	1052.51	350.84		
	Within "	59	690.15	11.70	1.7003	0.7100
Banner	Between blocks	3	2272.05	757.35		
	Within "	59	1440.25	24.41	1.7174	0.7100
Daubeney	Between blocks	3	3839.96	1279.99		
	Within "	59	934.72	15.84	2.1960	0.7100
Scott 1925 Abundance	Between blocks	3	570.11	190.04		
	Within "	59	3692.83	62.59	0.5502	0.7100
Banner	Between blocks	3	170.27	56.76		
	Within "	59	3428.80	58.12
Daubeney	Between blocks	3	15.79	5.26		
	Within "	59	4782.50	81.06
Scott 1926 Abundance	Between blocks	3	223.36	74.45		
	Within "	59	1020.24	17.29	0.7300	0.7100
Banner	Between blocks	3	1429.81	476.60		
	Within "	59	1981.82	33.59	1.3262	0.7100
Daubeney	Between blocks	3	239.20	79.73		
	Within "	59	1304.36	22.11	0.6414	0.7100
University of Alberta 1924 Abundance	Between blocks	3	4049.20	1349.73		
	Within "	44	17315.78	393.54	0.6169	0.7306
Banner	Between blocks	3	823.98	274.66		
	Within "	55	17188.56	312.52
Daubeney	Between blocks	3	745.83	248.61		
	Within "	60	4472.17	74.54	0.6022	0.7086
University of Alberta 1925 Abundance	Between blocks	3	642.35	214.12		
	Within "	59	11980.52	203.06	0.0252	0.7100
Banner	Between blocks	3	2020.94	673.65		
	Within "	60	3028.11	50.47	1.2912	0.7086
Daubeney	Between blocks	3	500.42	166.81		
	Within "	59	4323.47	73.27	0.4613	0.7100
University of Alberta 1926 Abundance	Between blocks	3	1479.22	493.07		
	Within "	60	3438.06	57.30	1.0762	0.7086
Banner	Between blocks	3	2832.67	944.22		
	Within "	59	3173.62	53.79	1.4327	0.7100
Daubeney	Between blocks	3	368.09	122.70		
	Within "	60	2091.01	34.85	0.6294	0.7086

pronounced again, though the values of Z obtained are not so great as those of 1924. At Macdonald College, on the other hand, evidence of considerable variation between blocks of similarly treated plots is apparent in all three years. Various other comparisons may be made from the results of Tables XXI and XXII, a study of which should be instructive for those interested in the problems of field experimental technique.

There can be little doubt, from these results, that at each of the stations certain comparisons between different rates of seeding of the same variety have been made on land differing significantly in general fertility level. The design of the experiment does not allow of any investigation into the possible differences in fertility between the areas on which different varieties were grown. In view of the results obtained in the foregoing analysis, however, the existence of these can hardly be regarded as unlikely.

Some of the possible effects of these soil differences upon the yield comparisons obtained have already been suggested. The question which naturally arises therefore is: whether some method cannot be employed to estimate the magnitude of any such effects and to eliminate this disturbing element from the experimental results.

An attempt was made to achieve this end, using the statistical procedure known as *partial regression*. The reasoning which lay behind the particular method adopted was somewhat as follows: It was assumed that the magnitude of the effect of differences in fertility level on the yield comparisons varied to some extent with the rate of seeding employed. Thus, if a higher level of fertility favored the higher rates of seeding, this effect might be expected to be most pronounced in the comparison "highest rate of seeding *vs.* check", and *vice versa*. Further, it was thought that in this connection the results of all three years, for any variety at any particular station, might well be considered together. In this way it was hoped to obtain a sort of average effect of rate of seeding upon yield, corrected for any observed systematic influence of fertility, whether due to soil differences or climatic factors.

A regression equation of the form

$$(y - \bar{y}) = a(x_1 - \bar{x}_1) + b(x_2 - \bar{x}_2) + c(x_3 - \bar{x}_3)$$

was obtained for each variety at each station. Here y represents the differences between the mean yield of the set of 16 plots of any particular seed rate and the mean yield of the adjacent 16 check plots. For each variety at each station there will be four such values of y each year, or twelve in all. The difference in seed rate between the "rate" plots under consideration and the adjacent check plots is represented by x_1 , while x_2 is simply x_1' . The "fertility factor" is represented by x_3 , which is the product of the deviation of the average yield of the 16 check plots in the particular "block" under consideration from the mean of the 12 such averages proper to the particular variety and station and x_1 . Symbolically

$$x_3 = (Y_c - \bar{Y}_c)x_1$$

where Y_c represents the mean yield of the 16 check plots in any particular

block. The mean of the 12 values of y is represented by \bar{y} ; the mean of the 12 values of x_1 by \bar{x}_1 ; and the mean of the 12 values of Y_c by \bar{Y}_c .

The values of the regression coefficients a , b , and c , appropriate to each variety at each station, were determined by the ordinary Least Squares solution. The results of this calculation are to be found in Table XXIII.

TABLE XXIII

PARTIAL REGRESSION COEFFICIENTS OF YIELD (BUSHEL PER ACRE) ON RATE OF SEEDING (BUSHEL PER ACRE) AND "FERTILITY FACTOR"

Station	Abundance			Banner			Daubeney		
	a	b	c	a	b	c	a	b	c
Charlottetown Macdonald College	.2739	.1056	-.0057	1.5182*	-.3803	-.0685	2.9217*	-.1167	-.0184
	-.2532	.0838	.0337	.5871	-.0457	-.0078	1.1524*	-1.3967*	.1064*
Scott University of Alberta	.4470	-.8840*	.0246	-.5920	-.7009	-.0148	.2260	.1831	.0018
	-.2038	-.1024	.0102	-.7457	-.1086	-.0567	1.9847*	-.4368	.0228

The significance of the various regression coefficients was tested by calculating the value of t , the ratio of the observed coefficient to its estimated standard error, and then determining from a table of "Student's" integral the probability of obtaining such a value of t by chance. Those coefficients which are to be regarded as statistically significant are indicated in Table XXIII by an asterisk.

It will be observed, on referring to the table, that there are altogether 7 significant values. Of these, 5 are associated with the variety Daubeney. Only one of the 12 coefficients c attained to the level of significance. It is however noteworthy that all the values of this coefficient obtained at Charlottetown are negative, whereas at the other stations Abundance and Daubeney give positive, and Banner negative values.

Having obtained the partial regression coefficients of yield on seed rate and on the "fertility factor", it is possible to investigate the relation between yield and seed rate under conditions of constant fertility. Putting then $(x_3 - \bar{x}_3)$ in each case equal to its expected value (which is of course conveniently zero) the theoretical values of y corresponding to different values of x_1 and $x_2 (=x_1')$ may be calculated, using the coefficients a and b of Table XXIII. This has been done, with the results shown diagrammatically in Fig. 5.

In considering the results portrayed in Fig. 5, two points should be borne in mind. Firstly the curves represent not the actual results of any particular year or years, but an attempt to estimate, from the experience of all three years, a set of results appropriate to the average condition of fertility obtaining at any particular station. Secondly, of the various regression coefficients a and b , only six may be regarded as differing significantly from zero. The standard

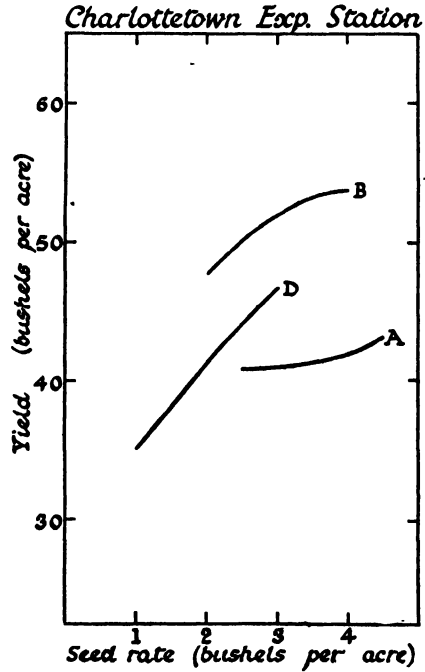
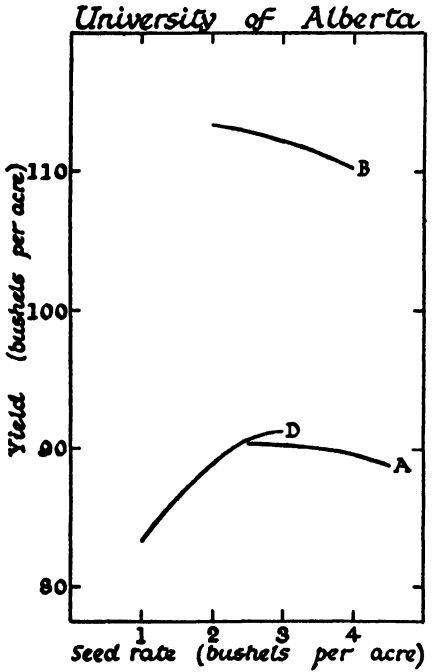
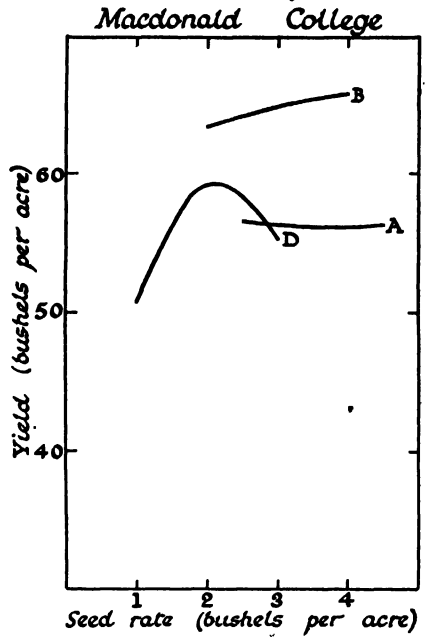
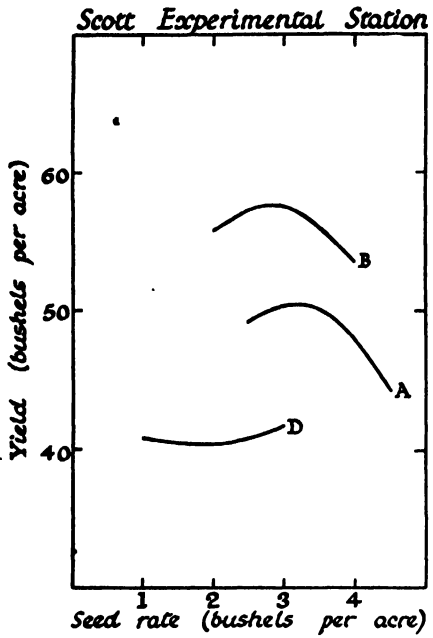


FIG. 5. Curves of partial regression of yield on rate of seeding. Three years' results. A = Abundance, B = Banner, D = Daubeney.

TABLE XXIV
STANDARD ERROR OF ESTIMATION OF
 $(Y - \bar{Y}) = a(x_1 - \bar{x}_1) + b(x_2 - \bar{x}_2) + c(x_3 - \bar{x}_3)$
APPROPRIATE TO THE CURVES OF FIG. 5

Station	Abundance	Banner	Daubeney
Charlottetown	1.69	2.79	2.62
Macdonald College	1.57	1.95	2.02
Scott	1.17	2.48	1.68
Univ. of Alberta	6.07	2.20	1.50

error, with which any particular point on the various curves is estimated, is given in Table XXIV.

The first feature to be noted in Fig. 5, as in Fig. 3, is that the optimum yields of the three varieties are in the order Banner, Daubeney,

Abundance at Macdonald College, Charlottetown Experimental Station, and the University of Alberta. At the last station, however, the difference in optimum yield between Daubeney and Abundance is quite insignificant. At Scott Experimental Station on the other hand Daubeney gives consistently lower yields than either of the other two varieties.

The response of Daubeney to increases in the rate of seeding at Macdonald College, the University of Alberta, and Charlottetown is also apparent. The absence of any such response at Scott is therefore the more noteworthy. It will however be observed that at this station the other two varieties show a definite tendency towards decreased yields at the higher seed rates. The optimum yield of Daubeney at Macdonald College when sown at approximately two bushels per acre is also clearly evident from the figure.

We may now attempt to consider the effect of these fluctuations in yield upon varietal comparisons. It is apparent that in general the optimum rate of seeding may differ for different varieties at the same station, and certainly does so for the same variety at different stations. In the neighborhood of the optimum, however, the yield is usually only slightly affected by changes in the seed rate. Furthermore, at all stations one variety, or more, exhibits no significant increase or decrease in yield at all over the range of seed rates explored.

These circumstances enable us to pick out a certain definite rate of seeding at each station, at which all three varieties may be sown and yet give an unbiased indication of their relative yielding capacities. Thus when the results of Fig. 5 are considered in the light of the standard error attached to any particular point on the various curves (see Table XXIV), it is at once apparent that at Scott a quite satisfactory comparison results if all varieties be seeded at the rate of 3 bushels per acre. Similarly at Macdonald College there would appear to be little loss of accuracy involved if all varieties were sown at the rate of $2\frac{1}{2}$ bushels per acre. At the University of Alberta, the fluctuations in yield between similarly treated plots of Abundance, and also of Banner, are so large that any effect upon yield comparisons resulting from seeding all plots at the rate of 3 bushels per acre would be relatively negligible. It is not possible to speak with such certainty in the case of Charlottetown, since at this station the maximum yield of Daubeney has clearly not been attained. It is possible however that at a seed rate of 4 or $4\frac{1}{2}$ bushels per acre all varieties

would be yielding sufficiently near to their optimum to enable a satisfactory varietal comparison to be made.

We are thus led to conclude that appropriate to each station there is a certain seed rate at which all three varieties may be sown and yet give yields which are truly comparable; the errors resulting from the varieties not all being seeded at their optimum rates being well within the ordinary experimental error as estimated from the fluctuations in yield of plots of the same variety. The particular seed rate to adopt would appear to vary from station to station, and is probably affected by both soil and climatic conditions.

The similar behavior of Abundance and Banner at any particular station is very apparent. This is interesting in view of the close similarity, previously noted, of these two varieties in size of seed and tillering behavior. As Daubeneey differs from the other two varieties in both of these characteristics it is impossible to separate their individual contributions to the observed results. The effect of tillering, however, would presumably be a tendency to stabilize the yield at different seed rates. In the case of Daubeneey this effect appears to have been masked by the counter influence of smallness of seed increasing abnormally the range in plant number.

Regression equations of an exactly similar nature were next determined, using however plant number as an independent variable instead of rate of seeding. The procedure was the same as that outlined above, except that x_1 is now the difference between the averages of the eight counts of plant number made on the "check" and "rate" plots of each block.

This undertaking had a twofold objective. First, to investigate the relationship between yield and stand of plants of the three varieties. Second, to see if the deviations from the rate of seeding regression equations producing the larger standard deviations of Table XXIV might not be to some extent explained by fluctuations in the percentage stand of plants obtained.

The values of the regression coefficients a , b , and c , obtained in this way, are given in Table XXV. Those which are to be regarded as differing significantly from zero are indicated by an asterisk. It will be observed that there are again seven such, but that these do not in all cases correspond to the seven significant coefficients of Table XXIII.

The goodness of fit of the two sets of regression formulas may be investigated by calculating the two quantities $\sum \sum (Y - y)^2$, where y is an observed yield value and Y the corresponding value calculated from the regression equation. The double summation sign indicates that the sums of the squares of the deviations from the twelve regression equations in each set are themselves to be summed. The resulting quantities are 647.83 in the case of the rate of seeding equations and 694.45 in the case of the plant number equations. The corresponding number of degrees of freedom is in both cases 92. We obtain by division, mean squares of 7.0416 and 7.5484 respectively, and find $Z=0.0347$. This value is quite insignificant. We therefore conclude that, taking the experiment as a whole, the goodness of fit of the two sets of regression equations is sensibly the same. Certainly there is no evidence of any superiority of those employing plant number as opposed to rate of seeding.

TABLE XXV

PARTIAL REGRESSION COEFFICIENTS OF YIELD (BUSHELS PER ACRE) ON PLANT
NUMBER (THOUSANDS PER ACRE) AND "FERTILITY FACTOR"

Station	Abundance			Banner			Dauboney		
	a	b	c	a	b	c	a	b	c
Charlottetown	.00001	.00184	-.00594*	.00842*	.00025	-.00641	.01264*	.00010	.00333
Macdonald College	-.00453	.00113	-.00224	.00522	.00140	-.00110	.00965*	-.00338*	.00222
Scott	-.00913	.00035	.00125*	-.00316	-.00150	-.00026	.00112	.00013	.00025
University of Alberta	-.00167	-.00141	.00253	-.00332	-.00274	-.00246	.00877*	-.00071	.00054

As in the preceding case, the coefficients *a* and *b* of Table XXV have been used to construct the curves of Fig. 6. Table XXVI gives the standard error appropriate to any point on the various curves.

TABLE XXVI
STANDARD ERROR OF ESTIMATION OF
 $(Y - \bar{Y}) = a(x_1 - \bar{x}_1) + b(x_2 - \bar{x}_2) + c(x_3 - \bar{x}_3)$
APPROPRIATE TO THE CURVES OF FIG. 6

Station	Abundance	Banner	Dauboney
Charlottetown	1.65	3.00	2.31
Macdonald College	1.64	2.07	1.60
Scott	2.03	2.34	1.66
Univ. of Alberta	5.84	3.68	1.21

The curves of Fig. 6 differ somewhat from those of Fig. 5. Two circumstances presumably account for this: differences in seed size between varieties, and the fact that the number of seeds sown bears by no means a constant ratio to the final stand of plants obtained.

Although the goodness of fit of the plant number regression equations is not significantly different from that of the rate of seeding equations, one point of considerable interest does emerge from these results. That is, that at three out of the four stations there are indications that the optimum stand of Dauboney, the small-seeded variety, may be greater than that of the other two varieties. At Charlottetown the optimum stand of both Dauboney and Banner does not appear to have been reached. Actually only at the University of Alberta can the observed difference in optimum stand be regarded as significant.

The various errors to which the determination of the plant number is subject have already been discussed. On account of these, the validity of the present results cannot be established above suspicion, even though the values actually used are all averages of eight individual counts. Nevertheless the results, such as they are, do not lend support to the principle of the Swedish system of variety testing noted in Section 1 of this paper.

A third set of regression equations were obtained, using the number of tillers per acre as a variable in place of plant number. The resulting coefficients are shown in Table XXVII.

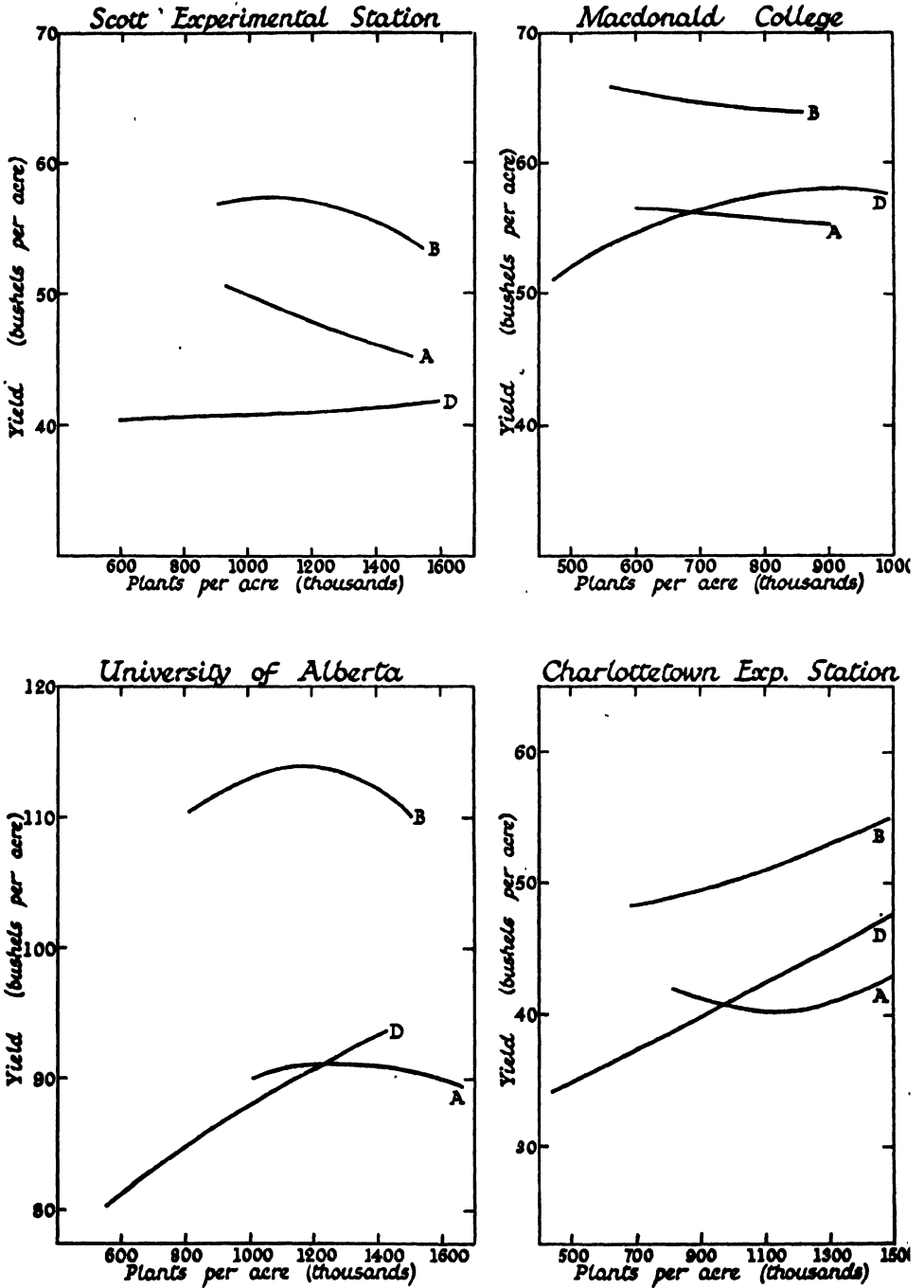


FIG. 6. Curves of partial regression of yield on plant number.
 'Three years' results. A = Abundance, B = Banner, D = Daubeney.

TABLE XXVII

PARTIAL REGRESSION COEFFICIENTS OF YIELD (BUSHELS PER ACRE) ON TILLER NUMBER (THOUSANDS PER ACRE) AND "FERTILITY FACTOR"

Station	Abundance			Banner			Dauboney		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
Charlottetown	.00562*	-.00064	.00043	.00718*	-.00206	-.00370	.01215	.00057	.00141
Macdonald									
College	-.00306	.00093	.00241	.00477	-.00177	-.00081	.00969*	-.00220*	.00396
Scott	.00016	-.00217*	.00144*	-.00332	-.00282*	-.00019	.00296	-.00019	.00093
University of Alberta	-.00478	-.00090	.00170	-.00259	-.00267	-.00201	.01520*	-.00041	.00259

The number of significant values is now eight. It will be observed that the linear coefficient *a* appropriate to Dauboney at Charlottetown has now fallen below, whilst the corresponding coefficients of the other two varieties have exceeded the level of significance. Otherwise, the results are very similar to those of Table XXV. The value of $S S (Y-y)^2$ is however now 800.03, indicating that the relation between yield and tiller number is in general less close than that between yield and either rate of seeding or plant number. The influence of tillering upon yield would therefore appear to have been somewhat less than might have been expected. Here again however the results must be accepted with a certain reserve, and should be regarded as indications only.

Comparing $S S (Y-y)^2$ in the case of the tiller number and rate of seeding equations, we find $Z=0.1042$. This is greater than the value obtained in the case of plant number and rate of seeding, but cannot however be regarded as significant.

The values obtained for the coefficient *c* have been in practically all cases insignificant when compared with their standard error. It is consequently possible that the effects of soil heterogeneity have been by no means entirely eliminated from the yield comparisons. The various coefficients *a* and *b* may therefore be affected by these to an extent of which their standard errors as calculated can give no valid estimate. This possibility should be borne in mind when considering the foregoing results.

Acknowledgment

The statistical examination of the data was undertaken whilst the author was enjoying the hospitality of the Statistical Laboratory, Rothamsted Experimental Station. Acknowledgment is due to Dr. R. A. Fisher, Head of the Department of Statistics, for his very valuable advice and criticism throughout the course of the work.

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THE EFFECT OF SUCROSE, COOKED POTATO, POTASSIUM BROMATE AND MALT UPON BAKING STRENGTH AT VARIOUS YEAST CONCENTRATIONS¹

By R. H. HARRIS²

Abstract

Three commercial and one experimental straight flour milled from 1931 Saskatchewan Marquis wheat were baked by a variety of methods, using various percentages of yeast.

Two basic formulas were used; No. 1 containing flour, water, salt and yeast, No. 2 containing the same ingredients plus sucrose. To these were added 40 cc. of cooked white potato extract and diastatic malt.

Using No. 2, no yeast starvation was apparent, but with No. 1 three flours showed this effect with higher yeast concentrations. The addition of potato extract resulted in pronounced yeast starvation with No. 1, and some starvation with No. 2. The further addition of malt produced optimum results with a yeast concentration of 3%.

A further series of bakings with another commercial flour (E) showed the improving effect of sucrose and malt in the presence of 20% of cooked white potato. Cooked sweet potato appeared to be able to support fermentation in the absence of added sucrose. Potassium bromate produced larger loaves with sugar and malt with high yeast concentrations.

As far as the flours used were concerned, using the basic formulas, there appeared to be no yeast starvation when 2.5% of sucrose was added (No. 2). When yeast stimulants were present, added sucrose and malt appeared to be necessary to prevent yeast starvation with a yeast concentration of 3%.

Introduction

A method for determining whether a baking procedure effected a separation of gassing power and flour strength has been described by Jorgensen (4). By this method, the author showed that only one of three baking procedures examined enabled an adequate distinction to be made between these flour properties. The failure of the two other test methods was due to a deficiency occurring in the supply of fermentable sugar before the loaf reached the oven, with consequent detrimental influence upon the loaf volume. Jorgensen (4) advocates a reduction in the 3% yeast content of the standard A. A. C. C. baking formula in order to offset the shortage of fermentable sugar which may arise in flours of relatively low diastatic activity. Jorgensen found the following formula quite satisfactory for the purpose of separating baking strength from diastatic activity:—yeast, 0.7%; salt, 1.25%; diastatic malt extract, 0.15%; water as per absorption; temperature of dough, 85°F. The dough time was approximately four hours, including the proofing period. The low yeast content of this formula would make a relatively small demand upon the fermentable sugar present in the fermenting dough.

Blish and Sandstedt (1), in a discussion of the differential baking test, recommended the use of 5% of sucrose in the formula when baking experimentally milled flours, to compensate for the lower diastatic activity of such

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flours and to ensure a plentiful supply of fermentable carbohydrate during the fermentation period.

Harris (2, 3) investigated the effect of sucrose upon a series of doughs, employing baking formulas which contained in one instance 3% of yeast, 2% of salt and 2.5% of sucrose. A second formula contained no sucrose but was otherwise identical with the above. The fermentation times and temperatures were in accordance with the standard method. During the course of this study it was found that, in the absence of sucrose, several of the flours produced very poor loaves, evidently as a result of yeast starvation. On the addition of an extract of cooked white potato to the doughs, the difference between the results obtained with and without added sugar was accentuated, probably as a result of yeast stimulation by the potato material without a compensating addition of fermentable carbohydrate. In the presence of sugar, satisfactory loaves were obtained, and the further addition of diastatic malt produced the largest loaves of the series. It was apparent that marked yeast starvation occurred in the absence of sucrose; it probably also occurred to a limited extent when potato extract and sucrose were used. A baking with 20% of cooked sweet potato added to the dough indicated that in the absence of sucrose this vegetable could apparently supply fermentable carbohydrate, in addition to acting as a stimulant to fermentation.

Potassium bromate in the absence of sucrose appeared to improve the loaf volume, except in the case of the two flours which showed the poorest response to the treatments with sucrose. In these yeast starvation evidently occurred. However, malt, in addition to potassium bromate and sucrose, increased the loaf volumes of these two flours, as well as the volumes of the higher protein flours.

In view, then, of the likelihood of yeast starvation occurring with higher concentrations of yeast, it seemed advisable to ascertain the effect of varying the quantity of this ingredient both above and below the standard 3% content. It was thought that this plan might give further information as to the role played by cooked potato extract in the fermentation process. Accordingly a series of bakings was carried out on several flours of different characters and baking strengths. The yeast concentrations used ranged from 1.5 to 7.5% (when sufficient flour was available). As a supplementary program, a study was made on a baker's patent, using cooked mashed potato, cooked sweet potato and malt and potassium bromate, to ascertain the effect of the various yeast concentrations upon the doughs containing these substances in addition to the regular baking formula.

Materials and Methods

The flours used in this investigation were all milled from Saskatchewan wheat of the 1931 crop. One sample, flour A, was experimentally milled from 2^o Marquis containing 10.7% of protein. Flour B was a commercially milled long patent from northern Saskatchewan wheat, while flour C was also a commercial flour produced in the central portion of the province. Flour D

was a strong break flour from a mill using Marquis wheat blends. Flour E, which was used in the further tests, was quite similar to C but possessed the advantage of being available in relatively large quantities. The protein and ash contents of these flours are shown in Table I.

The baking methods used in the present study were essentially those employed by the writer in experiments on the utility of cooked potato in baking (2, 3) and will not therefore be discussed in detail here. The simple formulas Nos. 1 and 2 consisted of a basic formula: flour, salt, water and yeast in No. 1, and these ingredients plus sucrose in No. 2. The yeast content was varied in both formulas while the amounts of the other ingredients were the same. The bakings with potato, bromate, etc. were made by adding these substances to the simple formulas. The potato extract was measured by volume and added to the flour just previous to mixing. The mashed potato was weighed directly into the flour. The potassium bromate, from a stock solution containing 1 gm. in 1000 cc. of water, was added by means of a Mohr pipette. Malt, when employed, was added in the form of an aqueous solution, 4 cc. of which contained 1 gm. of diastatic malt and 3.3 cc. of water. The yeast was measured in the form of a water suspension, containing 3 gm. yeast and 7.3 cc. of water in each 10 cc. of suspension.

The potato was prepared as described previously (2, 3), by boiling sliced potatoes in a galvanized vessel. The sole difference in the preparation of the potato extract and the mashed potato consisted in allowing the water to evaporate during cooking in the latter case. It was thought desirable to determine whether or not the effects of adding mashed potato or potato extract were similar.

Results

Loaf Volumes

The loaf volumes yielded by the four flours when baked by the various methods are shown in Table II. These results are calculated to a moisture basis of 13.5%. Comparing the volumes obtained with the simple formulas, Nos. 1 and 2, there appears to be little difference in the case of flour A, except for the bakings containing 6% of yeast. Flour B shows a slight tendency toward higher volumes for formula No. 1. Flour C reveals a decided increase with formula No. 2 when 3% or more of yeast is used, and sample D behaves in a very similar manner. The average loaf volume for the four flours baked with the two simple formulas shows an increase in three cases when sucrose was included in the formula. In one flour, B, there is no evidence of yeast starvation occurring at any yeast concentration, even without added sucrose. Flours C and D tend to give lower results in the absence of sucrose when more than 3% of yeast is added, but with sucrose present in the formula the loaf volumes yielded by these samples increase with increasing yeast concentration

TABLE I
PROTEIN AND ASH CONTENT
OF FLOURS USED

Flour sample	Protein %	Ash %
A	9.7	0.42
B	11.7	0.60
C	13.6	0.45
D	17.1	0.46
E	13.8	0.45

up to 6% of yeast. The addition of 2.5% of sucrose seems to prevent yeast starvation at any yeast concentration in any of the flours when baked with the simple formula.

TABLE II
LOAF VOLUMES OBTAINED BY THE USE OF INCREASING PERCENTAGES OF YEAST WITH
DIFFERENT BAKING FORMULAS (13.5% MOISTURE BASIS)

Yeast, %	Loaf volumes in cc.					Average all methods
	Simple formula No. 1	Simple formula No. 2	40 cc. Potato extract +		Formula No. 2 +2% of malt	
			Formula No. 1	Formula No. 2		
Flour A						
1.5	410	418	460	570	600	491
3.0	470	475	420	490	615	494
4.5	470	485	...	410	575	485
6.0	480	530	...	400	...	470
Average	457	477	440	467	597	
Flour B						
1.5	489	500	580	580	680	566
3.0	590	570	740	670	682	650
4.5	630	560	675	660	690	643
6.0	615	572	625	635	...	612
7.5	612	620	480	640	...	588
Average	587	564	620	637	684	
Flour C						
1.5	520	522	610	580	750	596
3.0	560	570	510	720	800	632
4.5	490	587	440	575	760	570
6.0	...	600	420	...	620	547
Average	523	570	495	625	732	
Flour D						
1.5	442	...	500	710	870	630
3.0	510	550	405	680	930	615
4.5	485	550	...	450	800	571
6.0	455	570	...	450	532	502
7.5	430	...	430
Average	473	557	452	544	783	
Average loaf volume for all four flours with all percentages of yeast						
	515	542	520	567	707	
Average loaf volumes for all four flours with various percentages of yeast						
Percentage yeast	1.5	3.0	4.5	6.0	7.5	
Loaf volume	571	598	567	533	509	

When the results obtained with the use of 40 cc. of potato extract are examined, a somewhat different picture is presented. Without added fermentable sugar, the loaf volume decreases with a yeast concentration greater than 1.5%, except in the case of flour B, which shows a small decrease after a yeast concentration of 3% is reached. The increased action of the yeast when stimulated by the potato material evidently accelerated the exhaustion of the available supply of fermentable carbohydrates. The addition of 2.5% of sucrose did not appear to alter materially the order of the loaf volumes, but did increase the size of the loaf throughout the series. A further addition of 2% of diastatic malt increased the loaf volumes generally, and shifted the maximum value to the 3% yeast concentration. These results would seem to indicate that with potato extract and 3% of yeast a plentiful and constant supply of fermentable carbohydrates during the fermentation period is desirable.

The average results for all the baking methods show that 3% of yeast gave the highest loaf volumes except in the case of the strong break flour, D, where only a slight decrease is noted in comparison with the value obtained with a yeast concentration of 1.5%. Apparently, then, no general yeast starvation occurred in the doughs containing 3% of yeast. These values are shown in Fig. 1. An examination of Fig. 1 shows that with all formulas, the relationship between mean loaf volume and yeast percentage is approximately linear with yeast concentrations greater than 3%, except flour B which shows the same trend beyond 4.5% of yeast. Flour A shows a poor loaf volume, due doubtless

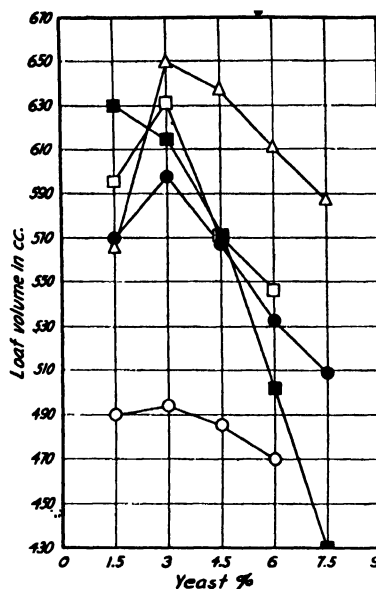


FIG. 1. Relation between mean loaf volume and yeast percentage. Legend: Mean, all flours, ●; Flour A, ○; Flour B, △; Flour C, □; Flour D, ■.

TABLE III
FORMULAS USED IN A SERIES OF EXPERIMENTS WITH FLOUR E

Series No.	Formula
1	Simple formula No. 1
2	Simple formula No. 2
3	Formula No. 1 + 20% of cooked white potato
4	Formula No. 2 + 20% of cooked white potato
5	Formula No. 2 + 20% of cooked white potato + 2% of malt
6	Formula No. 1 + 20% of cooked sweet potato
7	Formula No. 1 + .002% of potassium bromate
8	Formula No. 2 + .002% of potassium bromate + 2% of malt

to the extremely low protein content. Although sample D shows a decline after 1.5% yeast concentration, the relation does not approach a straight line function until the 3% point is reached.

The results discussed above agree with those obtained by the author (3) in that the addition of 40 cc. of potato extract accentuated the poor results obtained with those flours which yielded low loaf volumes in the absence of sucrose. It was shown also that sucrose and malt together with potato extract gave the greatest loaf volumes.

Baking Scores

A baking score was computed for the loaves baked from the flours, in the following manner:

Loaf volume.....	× 0.1	
Grain of loaf.....	× 1.0	Maximum value 10
Texture of loaf.....	× 1.0	Maximum value 10
Color of crumb.....	× 1.0	Maximum value 20

TABLE IV

LOAF VOLUMES AND BAKING SCORES YIELDED BY THE VARIOUS BAKINGS WITH FLOUR A

Yeast %	Loaf volume cc.	Color	Grain	Texture	Score
Simple formula No. 1.					
1.5	410	9.0	5	5	60
3.0	470	9.5	4	4	64
4.5	470	9.0	3	3	62
6.0	480	8.5	3	3	62
Simple formula No. 2.					
1.5	418	9.0	6.0	5.0	62
3.0	475	9.5	5.5	5.0	67
4.5	485	9.0	5.5	4.5	67
6.0	530	10.0	5.5	5.0	73
40 cc. Potato extract, simple formula No. 1					
1.5	460	12	5	5	68
3.0	420	10	4	4	60
40 cc. Potato extract, simple formula No. 2					
1.5	570	12.0	5.0	5	79
3.0	490	13.0	6.0	6	74
4.5	410	10.0	5.5	5	61
6.0	400	10.5	5.0	5	60
40 cc. Potato extract, simple formula No. 2, 2% of malt					
1.5	600	10.0	5.0	4	79
3.0	615	10.0	4.0	4	79
4.5	575	10.5	4.5	4	76

The color of the crumb was given a heavier weighting than the other interior characteristics on account of the popular demand for a white loaf. Symmetry of loaf was not considered in the present study.

In Tables IV, V, VI and VII the detailed and final baking scores for the four flours are shown. Table VIII summarizes the scores according to the percentage of yeast used in the baking, and Table IX summarizes Table VIII. These tables show a general tendency toward a decline in the score with a yeast concentration over 3%, and further strengthen the conclusions derived from a consideration of the loaf volumes alone. In other words, the optimum results were obtained with a yeast concentration of 3% in the entire series of bakings, with the exception of flour D which showed a slightly higher value with a yeast concentration of 1.5%.

The unbleached samples A and D reveal a slightly higher color score on the addition of potato extract.

Fig. 2 shows graphically the effect of increasing yeast concentration on the final baking score. A similarity is evident between the relationships depicted in this figure and that of loaf volume-yeast concentration shown in Fig. 1, the same tendency toward a straight line being shown with yeast concentrations greater than 3%. Flour A is very low, while sample B has the highest score throughout the yeast range.

The Effect of Cooked Potato

In order to determine the effect of cooked mashed potato in conjunction with various concentrations of yeast, further series of bakings were undertaken, using flour E. In each series the yeast concentration varied from 1.5 to 7.5%. The formulas used for these series are shown in Table III, and the results obtained on baking are shown in Table X.

It will be seen from an examination of Table X that series No. 1 showed a maximum loaf volume with a 3% yeast concentration, while in series No. 2, where sugar was added, the loaf volume increased up to a yeast concentration of 6%. This difference between the two series was of course due to yeast starvation in series No. 1.

The effect of adding cooked white potato is shown by the results of series No. 3 and 4. The loaf volume in both cases is greater than that from the analogous series (No. 1 or No. 2), with the exception of the 7.5% yeast value for series No. 3. This effect is not so marked in series No. 3, where the maximum loaf volume is obtained with a yeast concentration of 3%, as in series

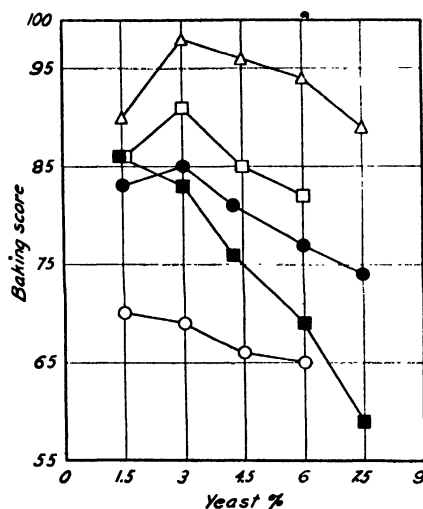


FIG. 2. Relation between mean baking score and yeast percentage. Legend: As in Fig. 1.

No. 4, where the largest loaf is obtained with 4.5% of yeast. This was no doubt due to yeast starvation brought about by rapid exhaustion of the available sugar, which exhaustion was in turn due to stimulation of the yeast by the potato.

Support for this hypothesis is given by the results of series No. 5, in which the plentiful supply of fermentable carbohydrate makes it possible to use a yeast concentration as high as 6%, and produces the largest loaf of all the bakings. The flour was apparently strong enough to stand the increased gas production.

TABLE V

LOAF VOLUMES AND BAKING SCORES YIELDED BY THE VARIOUS BAKINGS WITH FLOUR B

Yeast %	Loaf volume cc.	Color	Grain	Texture	Score
Simple formula No. 1					
1.5	489	20.5	8	8	85
3.0	590	21	8	8	96
4.5	630	21	6	6	96
6.0	615	21	4	5	91
7.5	612	19.5	5	5.5	91
Simple formula No. 2					
1.5	500	21.5	7	7.5	86
3.0	570	21	7	7.5	92
4.5	560	21	7	7.5	91
6.0	572	20.5	8	8	94
7.5	620	21	5	6	94
40 cc. potato extract, simple formula No. 1					
1.5	580	21	6	6.5	91
3.0	740	21	4	5	104
4.5	675	21	5	5.5	99
6.0	625	20	6	6	94
7.5	480	19	4	5.5	76
40 cc. potato extract, simple formula No. 2					
1.5	580	22	7	7	94
3.0	670	22	5	6	100
4.5	660	21	6	6	99
6.0	635	20.5	6.5	6.5	97
7.5	640	20	6.5	6.5	97
40 cc. potato extract, simple formula No. 2, 2% malt					
1.5	680	18	5	4	95
3.0	682	18.5	5.5	4	96
4.5	690	19	4	3	95

The effect of the addition of cooked sweet potato is shown in the results of series No. 6. Inasmuch as not only are the loaves larger throughout than in series No. 1, but also the loaf volume increases up to a yeast concentration of 4.5%, it is apparent that this material not only stimulates fermentation but also supplies fermentable carbohydrate.

The results of series No. 7 and 8 also show larger loaves than series No. 1 and 2, with maximum volumes at a yeast concentration of 4.5% of yeast.

Throughout all eight series, poor results were obtained with a yeast concentration of 7.5%, again due no doubt to yeast starvation which even malt and sweet potato could not prevent.

The use of 4.5% of yeast produced the largest loaves in five of the series (Nos. 2, 4, 6, 7, 8) while a concentration of 3%, the quantity prescribed by

TABLE VI

LOAF VOLUMES AND BAKING SCORES YIELDED BY THE VARIOUS BAKINGS WITH FLOUR C

Yeast %	Loaf volume cc.	Color	Grain	Texture	Score
Simple formula No. 1					
1.5	520	16	6	7	81
3.0	560	17	6	7	86
4.5	490	15	5	5.5	79
Simple formula No. 2.					
1.5	522	16	6	5	79
3.0	570	17	6	6	86
4.5	587	17.5	7	6	89
6.0	600	17.5	7	6.5	91
40 cc. potato extract, simple formula No. 1					
1.5	610	19	4	4.5	88
3.0	510	17	5	5	78
4.5	440	16	4.5	5	69
6.0	420	15.5	4.5	4	66
40 cc. potato extract, simple formula No. 2					
1.5	580	18.5	4	5	85
3.0	720	19.5	3	4	98
4.5	575	18	5	5	85
40 cc. potato extract, simple formula No. 2, 2% of malt					
1.5	750	16	4	4	99
3.0	800	17	4.5	4.5	106
4.5	760	18	4.5	4	102
6.0	620	17.5	5	5	89

TABLE VII
LOAF VOLUMES AND BAKING SCORES YIELDED BY THE VARIOUS BAKINGS WITH FLOUR D

Yeast %	Loaf volume cc.	Color	Grain	Texture	Score
Simple formula No. 1					
1.5	442	9.0	7.5	6	67
3.0	507	11.5	7.0	7	76
4.5	485	9.0	6.0	7	70
6.0	455	9.0	6.0	7	67
Simple formula No. 2					
3.0	550	11.0	7.0	7.0	80
4.5	550	12.0	7.0	7.0	81
6.0	570	12.5	7.5	7.5	84
40 cc. potato extract, simple formula No. 1					
1.5	500	10.5	5	5	70
3.0	435	10.0	3	4	60
40 cc. potato extract, simple formula No. 2					
1.5	710	15.0	7	7.0	100
3.0	680	12.0	6	6.0	92
4.5	450	10.0	4	3.0	62
6.0	450	9.5	4	3.5	62
7.5	430	9.5	4	3.0	59
40 cc. potato extract, simple formula No. 2, 2% of malt					
1.5	870	12.0	3.0	4	106
3.0	930	11.0	1.5	3	108
4.5	800	10.5	1.0	2	93
6.0	532	10.5	0.5	1	65

TABLE VIII
SUMMARY OF SCORES ACCORDING TO YEAST PERCENTAGE

Yeast %	Color	Grain	Texture	Final score	Yeast %	Color	Grain	Texture	Final score
Flour A					Flour C				
1.5	10.4	5.2	4.8	70	1.5	17.1	4.8	5.1	86
3.0	10.4	4.9	4.6	69	3.0	17.5	4.9	5.3	91
4.5	9.6	4.7	4.1	66	4.5	16.9	5.2	5.1	85
6.0	9.7	4.5	4.3	65	6.0	16.8	5.7	5.1	82
Flour B					Flour D				
1.5	20.6	6.6	6.6	90	1.5	14.9	5.5	5.5	86
3.0	20.7	5.9	6.1	98	3.0	14.9	5.1	5.3	83
4.5	20.6	5.6	5.6	96	4.5	14.4	5.0	4.9	76
6.0	20.5	6.1	6.4	94	6.0	14.3	5.6	5.1	69
7.5	19.9	5.1	4.7	89	7.5	14.7	4.5	3.8	59

the official A. A. C. C. method, produced the largest loaf in two series only, this being due to the low fermentable carbohydrate content of these series.

It is of interest to note that series No. 7 and 8 produced comparatively large loaves with high yeast concentrations.

These results are in agreement with those previously obtained by the author (2, 3).

The substitution of 20% of white cooked potato for the 40 cc. of white potato extract appeared to give strong stimulation. This effect was most marked in the absence of sucrose.

TABLE IX

AVERAGE SCORES OF ALL BAKINGS WITH THE FOUR FLOURS ARRANGED ACCORDING TO INCREASING CONCENTRATION OF YEAST

Yeast %	Color	Grain	Texture	Final score
1.5	14.9	5.5	5.5	83
3.0	14.9	5.1	5.3	85
4.5	14.4	5.0	4.9	81
6.0	14.3	5.6	5.1	77
7.5	14.7	4.5	3.8	74

TABLE XII

MEAN LOAF VOLUMES AND BAKING SCORES FOR THE VARIOUS YEAST CONCENTRATIONS USED IN SERIES 1 TO 8 WITH FLOUR E

Yeast %	Mean loaf volume cc.	Mean baking score
1.5	592	88
3.0	662	92
4.5	681	94
6.0	642	88
7.5	583	80

The baking scores assigned the loaves in these series of bakings are, with the exception of series No. 6, shown in Table XI. Series No. 6 has been omitted because of the exceedingly low color, grain and texture score. Considerable variation in crumb color is evident among the loaves produced by the different methods. The addition of sucrose and cooked potato (Series No. 4), or sucrose, malt and cooked potato (Series No. 5) appeared to reduce the color score. The bromate bakings without malt (series No. 7) yielded the highest color score for the series, but the grain and texture were poor. The inclusion of sucrose (series No. 2, 4,) and sucrose and malt (Series No. 5, 8) caused the maximum baking score to fall at a yeast concentration higher than 3%, the

TABLE X

LOAF VOLUMES OBTAINED IN THE BAKINGS OF SERIES 1 TO 8 WITH FLOUR E

Yeast %	Series No.*							
	1	2	3	4	5	6	7	8
1.5	530	522	600	640	665	565	600	615
3.0	600	572	750	720	720	640	670	625
4.5	560	587	725	775	750	695	690	670
6.0	470	586	580	762	810	645	620	660
7.5	440	550	500	680	695	605	570	630

* See Table III. Simple formula No. 1 includes flour, water, salt and yeast, simple formula No. 2 includes flour, water, salt, yeast and sucrose.

TABLE XI

BAKING SCORES ASSIGNED THE LOAVES BAKED IN SERIES NO. 1 TO 8, WITH FLOUR E

Yeast, %	Loaf volume cc.	Color	Grain	Texture	Score
Series No. 1					
1.5	530	17.0	7.5	8	85
3.0	600	18.0	7	6	91
4.5	560	17.5	7.5	7	88
6.0	470	16.0	5	5	73
7.5	441	15.0	5	3	67
Series No. 2					
1.5	522	17.0	7	7.0	83
3.0	572	17.5	7	7.5	89
4.5	587	18.0	7	8.0	92
6.0	586	17.0	6	8.0	90
7.5	550	17.5	6	6.0	84
Series No. 3					
1.5	600	16	8	8	92
3.0	750	18	6	6	105
4.5	725	19	5	6	102
6.0	580	15	6	7	86
7.5	500	14	5	7	76
Series No. 4					
1.5	640	12	7	8.0	91
3.0	720	15	5	7.0	89
4.5	775	14	4	6.0	101
6.0	762	13	4	5.5	98
7.5	680	12	4	5.5	89
Series No. 5					
1.5	665	11	3	3	83
3.0	720	10	3	2	87
4.5	750	10	4	3	92
6.0	810	10	3	3	97
7.5	695	10	4	4	87
Series No. 7					
1.5	600	19	8.5	9.0	96
3.0	670	20	9.0	8.0	104
4.5	690	20	6.0	6.0	101
6.0	620	18	5.0	6.0	91
7.5	570	17	5.0	6.5	85
Series No. 8					
1.5	615	12.0	5.5	5	84
3.0	625	11.0	5.0	5	83
4.5	670	13.0	3.0	3	86
6.0	660	10.0	2.0	3	81
7.5	630	9.5	1.0	1	74

optimum for the bakings without these ingredients (Series No. 1, 3, 7). The mean loaf volume and score for all the methods show the optimum yeast concentration to be 4.5%. These values are shown in Table XII.

General Summary and Conclusions

1. There was no apparent yeast starvation when a series of four flours were baked with the addition of 2.5% of sucrose at any yeast concentration used in this study. Without sucrose, detrimental effects upon the loaf were evident at the higher yeast concentrations for three of the flours.

2. Potato extract caused yeast starvation at higher yeast contents in the absence of added sugar. This effect persisted to a lesser degree in the presence of 2.5% sucrose. The further addition of 2% diastatic malt obviated yeast starvation with 3% of yeast for all the flours. The highest mean loaf volume and baking score of any method was given by such a formula. From these results, sucrose and diastatic malt in the concentrations used would appear to be necessary if yeast starvation is to be avoided with a yeast concentration of 3%.

3. Cooked white potato served to support fermentation somewhat better than potato extract in the absence of added sugar. Sucrose, or sucrose and malt, however, effected a progressive increase in loaf volume and shifted the maximum loaf volume to a higher yeast concentration.

4. Cooked sweet potato appeared to supply fermentable carbohydrate as well as exerting a stimulating effect upon fermentation, though this stimulating effect would seem to be less than the somewhat similar action of cooked white potato.

5. Potassium bromate yielded very similar results with and without sucrose and malt with the exception that somewhat better results were obtained with very high yeast concentrations.

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REPLACEABLE BASES, HYDROGEN AND BASE-HOLDING CAPACITY OF ALBERTA SOILS¹

BY N. HOLOWAYCHUK²

Abstract

Studies on soils from three major soil groups in Alberta showed relatively high content of replaceable bases with normal calcium, magnesium and sodium-potassium ratios. The A_2 horizon of the wooded soils was lowest in replaceable bases and indicates excessive leaching. Excessive leaching has not occurred in any of the horizons of the brown and the black soils. Losses by leaching in the brown and black soils have resulted mainly from the movement of water-soluble cations.

Movement of base-exchange complexes from the A_2 horizon of the wooded soils was apparently due to dispersion rather than disintegration. The excess SiO_2 in the A_2 horizon has resulted from disintegration of feldspars rather than from the breaking up of the base-exchange complex.

Greater proportions of hydrolytic acidity were found in the A_2 and B_1 horizons of the wooded soils than in the black soils.

The wooded soils appear to belong to the podsol group according to the Gedroiz system of classification.

Introduction

The question of base-exchange in soils has been given considerable attention, especially in the last decade, and a good deal of work has been done at various institutions on this continent and in Europe. It is the intention of the writer to show in this report how the soils of Alberta compare with each other and with those from other parts of the world.

Way was the first to report on base-exchange in soils, and his findings are well summarized by Hissink (4). Following this, little work was done, or at least made available, until about 1920 when the works of Hissink and Gedroiz were translated. Since then an ever-increasing number of workers have taken part in these investigations, resulting in an extensive literature.

The study of base-exchange in soils as developed by present-day workers seems to resolve itself into the following four lines of activity:

(1) The study of the chemistry of ion exchange in soils. (2) Isolation and identification of the soil fractions having base-exchange properties. (3) The study of the effect of the dynamic forces of the soil on base-exchange properties of the soil. (4) The study of the agronomic significance of base-exchange in soils.

A striking feature of base-exchange reaction is the rapidity with which the equilibrium is reached. These are apparently surface reactions in which all the exchangeable ions are easily accessible. Hissink and Gedroiz (3, 4) explain base-exchange as physico-chemical rather than non-polar absorption or the double decomposition of an insoluble salt. These however may occur to some extent. Both Gedroiz and Hissink looked upon the exchange complex as being an insoluble anion (a colloidal particle) with an electrical double layer where the cations are held, partially adsorbed on the surface and

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partially as an atmosphere of dissociated ions. This concept is generally held with respect to base-exchange, and the base-exchange properties of the soil are attributed to the colloidal fraction, organic or mineral. The properties of the soil, such as dispersion, flocculation, swelling, structure, etc., can be explained by observing the effect of the exchangeable cation of the electrical double layer. It is found that cations of low molecular weight and valency are more hydrated and have a higher osmotic pressure, thus increasing the dispersion, swelling, etc., of the soil. These effects are decreased as the cation increases in valency and molecular weight.

The isolation and identification of the complex showing base-exchange properties have been given considerable attention by the workers in this field. Way was the first to notice that the seat of base-exchange reactions was in the clay fraction of the soil. Gedroiz and Hissink have attributed this property to certain alumino-silicates in the clay fraction, and have also found that the "humus" or "humic" portion of the soil is very important in this respect. They found also a very striking similarity between this exchange complex in the soil and ordinary zeolites, and this fact has led many workers to adopt the view that base-exchange in the soil was due, in part at least, to natural zeolites. Kerr (8) more recently was able to extract from the soil a mineral fraction which was mainly responsible for base-exchange in the mineral portion. He compared this fraction with zeolites as to exchange equilibria, stability and specific gravity and found no correlation. Analysis of this fraction by Truog and Chukka (15) on further purification, showed that it is an alumino-silicate with an Al_2O_3 : SiO_2 ratio of about 1: 4. Kelley, Dore and Brown (7) further investigated this point by subjecting natural zeolites, bentonites and soil colloids to X-ray and stability studies. They found no resemblance in the X-ray spectra of zeolites and soil colloids but a resemblance was noticed in the bentonites and soil colloids. Also, the soil colloids and bentonites were more or less alike; in regard to stability, when subjected to heating, both being much more stable than zeolites. Presumably the base-exchange complex is an hydrated alumino-silicate, rather complicated in structure, and is one of the secondary stages of weathering of rocks.

High base-exchange capacity of organic soils has led some workers to investigate this field. McGeorge (10) found a high correlation between the lignin fraction and base-exchange capacity. There was less correlation when cellulose and hemi-cellulose were compared.

Important dynamic forces such as the climate and water movements act on the soil. Thus, where the climate is cool and moist considerable organic matter is produced having a wider carbon-nitrogen ratio than is found in the more arid regions. Decomposition under a cool moist climate is slow and likely to produce acid end-products. The soil solution in this case tends to become more acid than it would under drier and warmer conditions. Thus, the reaction of the soil solution as well as its movements and quantity are important in determining the development of the soil, and as the cations exert a marked influence on the physical properties of the soil colloids, the effect of the soil solution on these cations should be considered.

Gedroiz (3) noticed that if a soil was leached with water the basic cations were gradually removed and gave rise to an acid complex. He found that this was somewhat more dispersed than where calcium was the main complex and tended to move with the general water movements of the soil. He also found that if this complex was sufficiently acid it would disintegrate into its oxides—mainly aluminium oxide and silicon dioxide. Thus the hydrolytic action of pure water tends to produce what is essentially a process of podsolization, and this action is increased if the soil is made acid by decomposing organic matter as mentioned previously.

Where the alkali cations predominate, *e.g.*, sodium, the colloids are in a dispersed state. As the sodium complex hydrolyzes easily when not prevented by the common-ion effect of sodium salts, the resulting sodium hydroxide is leached away and an acid complex (soloti) remains.

Gedroiz has actually worked out a system of soil classification based on the degree of saturation and also on the basic cation content of the soil. This system is given by Afanasiev (1) and contains two large groups, *viz.*, podsoles and laterites where the complex is unsaturated with basic cations, and chernozems and alkaline where it is saturated.

Gedroiz and Hissink were the first to develop the idea of unsaturation. The exchange complex is said to be unsaturated when some of the exchangeable cations are hydrogen. The degree of saturation is the total quantity of basic cations compared to the total capacity of the complex for cations.

Base-exchange has a significant role in agriculture. Soil reaction, soil structure and availability of cations are more or less directly connected with base-exchange. In considering soil structure Hissink (4) found that soils with 35% of replaceable, univalent cations had poor tilth but were satisfactory if these cations were reduced to 20%. Somewhat similar results are produced by unsaturation. Turner (16) working in Trinidad has estimated that poor conditions are produced where the unsaturation is about 40%. Soil acidity is also directly affected by base-exchange. In this respect, the degree to which the complex ionizes and the ratio of hydrogen to basic ions determine the active acidity. The question of unsaturation and availability of cations has been dealt with by Robinson and Williams (14). They found that available calcium varies directly as the exchangeable calcium and unsaturation of the soil. Where exchangeable calcium was high, the question of unsaturation was less vital. Pierre (13) was able to obtain a fair correlation between plant injury and soil unsaturation. It may be possible that the toxicity of the more acid soils is due to certain elements, for example, Kelley and Brown (6) found replaceable aluminium, manganese and iron in some acid soils. Fertilization of soils is also influenced by base-exchange. Kelley (5) has discussed cases in which some difficulty was experienced in getting potassium down to deep-rooted plants. In these cases potassium was fixed in the surface horizons. The ammonium radical is similarly fixed when ammonium salts are applied. In all these cases the predominant exchangeable cation must be taken into account. The more fertile soils are high in replaceable bases with calcium as the dominant cation.

The data and discussion given in this paper have been planned to compare the Alberta soil groups in regard to the vertical distribution of their replaceable bases, base-holding capacity, and replaceable and hydrolytic acidity. Comparisons are also made with soils reported from Illinois and Rothamsted.

Description of Alberta Soils

The selection of samples used in this investigation has been such that they represent the three major soil groups found in Alberta. These soils are grouped according to the kind and degree of weathering to which they have been subjected. The extent and analysis of these groups have been reported on by Wyatt and Newton (17) and Leahey (9).

In the south of the province there are the brown soils, which are the result of weathering under low precipitation and high evaporation. Such conditions are not conducive to excessive growth and accumulation of organic matter, hence the quantities of organic acids produced will not be very great. It may be seen also that with low precipitation and high evaporation there will be very little water passing downwards. Most of it penetrates to a shallow depth and returns to the surface by evaporation and transpiration. The absence of percolation accounts for accumulation of salts near the surface. Under these conditions there will be little or no movement of soil colloids. The exchangeable complex present in these soils is mainly mineral and very little organic matter is present.

The next distinct soil group consists of the black soils. These are produced under moister conditions than were the brown. Here the evaporation is less, but the downward movement of water is still not excessive. This climate is conducive to active growth and grass is usually the main covering. Thus it may be seen that only the more soluble parts will be moved. There has been a movement of electrolytes, such as calcium carbonate, but this has not been excessive, and as a rule there is sufficient to hold the soil colloids.

The third group consists of wooded soils, composing about two thirds of the area of the province. They are the least fertile of the groups and are produced under more humid conditions than either of the other two. These humid conditions may be brought about by higher precipitation, lower evaporation, poor drainage or a combination of these. The vegetation in this area is fairly rank, consisting mainly of trees, shrubs, moss and some grass. The soil is maintained in a humid condition, thus providing water for the leaching process. This water is slightly acidic, mainly from carbon dioxide produced by the decay of organic matter. The leaching power of the water is increased, and thus there is a downward movement of the bases released by the acid conditions. The removal of bases makes the soil colloids less stable. Thus there is a horizon in the upper part of the soil where a removal of bases and colloids has occurred, with subsequent precipitation farther down if the soil has been weathered long enough.

The precipitation in Alberta is usually between 10 and 20 in., hence the differences in soils have been brought about mainly by differences in evaporation.

The movement of bases and colloidal fractions in the above soils should be indicated by the content of replaceable bases and the base-holding capacity of the various horizons.

In general the soil profile is characterized by three general horizons, designated *A*, *B* and *C*. The surface horizon *A* has undergone some weathering. Below this there is horizon *B* showing an accumulation of degradation products from *A*. The more or less unaltered horizon underlying *A* and *B* is designated by *C*.

This general grouping of soil horizons is frequently subdivided further, as follows: *A*₀, the layer of raw or partially decayed organic matter; *A*₁, the horizon of accumulation of decayed products from *A*₀, if these are not leached away; *A*₂, the horizon where the leaching has occurred to greatest extent; *A*₃, the horizon of transition between *A*₂ and *B*₁; *B*₁, the horizon of accumulation of colloidal matter from *A* horizons; *B*₂, the horizon of precipitation of electrolytes from above, mostly as carbonates; *C*, the horizon which has not undergone any appreciable weathering.

Five profiles were taken from the wooded area, three from the black soils and two from the brown. Those from the wooded area were taken at Fort Vermilion, Ksituan (near Spirit River), Breton, Wabamun and Alberta Beach, those from the black soil area were from Edmonton and Hobbema, and the two brown profiles were taken at Shouldice and Benton.

Experimental

The methods of extraction employed in base-exchange work may be classified into three groups: (1) Treatment of the soil with a neutral salt; (2) Treatment of the soil with a dilute acid; (3) Electrodialysis.

Ammonium acetate was used as the extracting solution and so the method belongs to the first group. There are two distinct advantages in using ammonium acetate as the extracting solution: (1) ammonium acetate is a neutral salt and has pronounced buffer properties around pH 7; (2) it is easily expelled by a single ignition.

However a very considerable amount of work has been done using ammonium chloride, so it was thought advisable to compare these two salts. A series of samples was run using each solution and the replaceable calcium and magnesium determined. The results, shown in Table I, were consistent throughout, and no important difference was noticed between the two salts.

The procedure of extracting a soil sample with the salt solution and the subsequent determination of the bases replaced were as follows: the air-dry soil was mixed and a portion of it crushed to pass a 20-mesh sieve. Twenty-five grams of the sifted soil was weighed out and digested overnight with 500 cc. of *N* ammonium acetate (pH 7). The sample was filtered the following day on a Buchner using suction, and then leached with more ammonium acetate till a litre of the solution was obtained.

A 100-cc. sample (or larger if the base contents were low) of the solution was evaporated on a steam bath. A little nitric acid was added and, when dry, the sample was ignited over a Meker burner. The residue was dissolved in hot water with

the addition of 2-3 cc. of hydrochloric acid, and ammonia separation carried out to remove aluminium and iron if present. Calcium was precipitated in the filtrate from the latter as oxalate and titrated with 0.1 *N* potassium permanganate. The filtrate from the calcium determination was evaporated and ignited to remove all ammonium salts. Following this the residue was evaporated two or three times with sulphuric acid, ignited at about 700°C., and then the sulphate held by bases determined as barium sulphate. The sulphate expressed in equivalents represents mostly the magnesium and sodium-potassium that have been replaced*. Magnesium was determined in a separate quantity of solution by Epperson's method (Treadwell and Hall) after aluminium, iron and calcium were removed as indicated above. The base-holding capacity was determined by leaching 25 gm. of air-dry soil with a litre of *N* calcium chloride on a Buchner. Following this it was washed free of chlorides and then the calcium fixed was replaced and determined as in the regular method.

Hydrolytic acidity was determined by shaking 25 gm. of soil with 250 cc. of normal calcium acetate for one hour, filtering and titrating with 0.1 *N* potassium hydroxide. Phenolphthalein was used as indicator.

Replaceable acidity was determined by shaking 25 gm. of soil with 125 cc. of normal potassium chloride for one hour, filtering and titrating with 0.1 *N* potassium hydroxide using phenolphthalein as indicator. This method was checked by using 250 cc. of normal barium chloride in place of potassium chloride. The results obtained are shown in Table II and did not show any pronounced differences.

There is a marked removal of replaceable bases in the *A*₂ horizon of the wooded profiles. Of these, the Alberta Beach profiles show the least leaching, but this does not represent the most degraded of the author's profiles. The *A*₁ horizon of this profile has the least replaceable bases, but it has fair structure

TABLE I
REPLACEABLE CALCIUM AND
MAGNESIUM OBTAINED BY AMMONIUM
CHLORIDE AND AMMONIUM ACETATE
METHODS*

Calcium		Magnesium	
NH ₄ Ac	NH ₄ Cl	NH ₄ Ac	NH ₄ Cl
<i>Breton B₁</i>			
18.8	19.2	6.7	9.0
18.8	19.0	7.0	8.8
17.6	18.5	6.5	8.7
17.6	18.6	6.3	9.2
<i>Breton A₂</i>			
7.5	7.1	1.5	1.5
7.6	6.8	1.4	1.7
7.6	6.8	1.5	1.5
7.7	—	1.5	—
<i>Breton A₁ and A₀</i>			
25.5	23.0	4.6	4.9
25.2	23.9	4.8	5.1
24.5	22.5	4.9	4.7
24.1	22.8	4.9	4.7
<i>Edmonton A₂</i>			
24.7	28.3	8.4	8.9
25.2	28.5	9.0	8.7
<i>Edmonton B₁</i>			
18.8	18.0	9.5	7.1
18.8	19.4	8.8	7.5
17.1	20.1	9.3	6.9
22.6	19.6	9.4	6.5

*Results expressed as milli-equivalents per 100 gm. of soil.

*This method is not very satisfactory for the determination of small quantities of sodium-potassium. However, it gives a fair indication when appreciable quantities are present.

and some organic matter incorporated in it. The A_2 horizon in this profile also lacks the extremely bleached color found in the corresponding horizon of the other wooded profiles.

TABLE II
REPLACEABLE BASES, HYDROGEN AND BASE-HOLDING CAPACITY OF ALBERTA SOILS

Horizon	Depth, in.	Calcium	Magnesium	Total bases	Sodium, potassium etc.	Base holding capacity	Hydrogen		Ratio ₁ †	Ratio ₂ ‡	Ratio ₃ §
							Replaceable	Hydrolytic			
Breton (wooded)											
A ₀ A ₁	0—2	24.8	4.9	29.8	0	30.4	0	6.1	99	100	83
A ₂	2—18	6.7	2.3	8.7	0	10.5	0	2.2	83	100	80
B ₁	18—42	15.1	6.5	20.3	0	28.9	1.8	5.5	70	92	78
C	At 84	41.5	5.7	46.7	0
Wabamun (wooded)											
A ₀	1	37.8	5.9	43.7	0	46.8	0	93	99
A ₁	1—3	11.2	2.8	14.0	0	12.3	0	.8	114	100	95
A ₂	3—12	8.7	3.0	12.1	0	9.2	0	1.7	132	100	88
B ₁	12—60	17.8	7.0	25.5	0	22.7	1.5	4.8	112	96	84
B ₂	At 60	72.5	7.2	79.9	0
Ft. Vermilion (wooded)											
A ₁	0—3	20.0	8.0	31.7	3.7	25.5	.2	124	99
A ₂	4—8	3.7	2.1	7.2	1.4	15.0	2.3	6.0	50	76	55
B ₁	8—18	14.8	13.8	35.8	7.0	42.2	0	1.0	83	100	97
B ₂	At 27	100.0	11.3	112.2	.9
Ksituan (wooded)											
A ₀ , A ₁	0—5	35.6	5.7	41.3	0	40.0	0	102	100
A ₂	5—12	7.5	2.7	10.2	0	9.5	.9	3.0	108	92	77
A ₂	12—22	14.1	10.2	24.5	0	28.5	4.5	7.0	87	84	77
B ₁	22—27	18.2	12.3	30.9	0	28.9	0	1.7	107	100	95
B ₂	At 30	46.3	12.0	56.3
Alberta Beach (wooded)											
A ₁	2—5	11.7	3.9	17.6	2.0	19.0	0	4.1	93	100	81
A ₂	5—12	9.9	3.5	15.2	1.9	12.3	0	1.9	123	100	89
A ₂	12—18	12.0	3.7	15.9	.2	16.3	0	1.7	98	100	90
B ₁	At 36	14.4	5.9	21.6	1.3	18.6	0	1.7	116	100	91
B ₂	At 72	56.0	5.8	63.2	1.4
C	At 72	48.3	8.0	56.7	.3
Edmonton 634 (black)											
A ₁	1—12	50.0	6.4	57.4	1.0	51.0	0	112	100
A ₂	12—24	30.5	8.9	41.4	2.0	37.0	0	1.1	110	100	98
B ₁	24—30	27.5	8.2	35.7	0	32.4	0	.7	112	100	98
B ₂	30—48	73.0	6.3	79.3	1.5
C	At 72	67.0	9.1	76.1	0.7
Hobbema (black)											
A ₁	0—12	31.0	10.0	39.9	0	45.6	.2	6.5	87	99	86
A ₂	12—16	15.9	6.7	22.2	0	25.4	.0	2.6	88	100	89
B ₁	16—30	18.4	8.7	27.3	0	29.7	.0	1.2	92	100	96
B ₂	30—36	90.7	9.1	100.2	0
C	60—72	28.5	9.4	37.2	0
Edmonton 649 (black)											
A ₁	0—5	34.0	7.1	42.2	1.0
A ₂	5—13	25.0	8.7	31.6	0
B ₁	13—27	18.0	9.2	26.6	0
B ₂	27—42	66.5	10.0	74.0	0
C	At 48	22.0	9.1	30.0	0

TABLE II—Continued

Horizon	Depth, in.	Calcium	Magnesium	Total bases	Sodium, potassium etc.	Base holding capacity	Hydrogen		Ratio ₁ †	Ratio ₂ ‡	Ratio ₃ §
							Replaceable	Hydrolytic			
Benton (brown)											
A ₁	0—5	26.5	5.4	33.7	1.8	41.8	Alk.	.3	81	100+
A ₂	5—10	20.3	5.7	28.3	2.3	22.5	Alk.	0	127	100+
B ₁	10—12	25.8	6.9	33.6	.9	25.3	Alk.	136	100+
B ₂	12—24	76.7	11.9	89.0	.4
C	At 48	48.0	12.1	62.0	1.9
Shouldice											
A ₁	0—7	58.0	6.0	64.0	0	60.5	Alk.	106	100+
A ₂	7—17	115.0	5.1	120.0	0	93.0	Alk.	140	100+
B ₁		88.5	10.5	99.0	0	95.0	Alk.	104	100+
B ₂		95.5	23.1	121.5	2.9

*Results expressed as milli-equivalents per 100 gm. of soil, †Ratio₁ = $\frac{\text{total bases}}{\text{base-holding capacity}} \times 100$,

‡Ratio₂ = $\frac{\text{total bases}}{\text{total bases} + \text{replaceable hydrogen}} \times 100$, §Ratio₃ = $\frac{\text{total bases}}{\text{total bases} + \text{hydrolytic hydrogen}} \times 100$.

The most significant difference found in replaceable-base content of the different soils was in the A₂ horizon. In the better developed wooded profiles it was between 7.2 and 12.1 M.E. (milli-equivalents) per 100 gm. of soil. In the black soils this was between 22.2 M.E. (Hobbema) and 41.4 M.E. (Edmonton). There is no apparent leaching in this horizon in the brown soils and as a matter of fact, an accumulation occurs in Shouldice profile.

There are no great differences in the replaceable base contents of the B₁ horizons of any of the profiles studied, but in the wooded soils, the replaceable bases in this horizon are at least double the total replaceable bases found in the A₂ horizon.

The wooded profiles taken individually show a replaceable base content in the A₀ horizon that compares well with that of the A₁ horizon from the black soils. The A₂ horizon shows a decided removal of replaceable bases when it is compared with the other horizons of the same profile, or with the A₂ horizon of the black or brown soils.

The black-soil profiles, with the exception of the A₂ horizon, show a replaceable base content somewhat similar to the wooded profiles. The A₂ horizon of the black soils shows no pronounced removal of bases as it does in the wooded profiles.

In case of the brown soils there is no movement of replaceable bases indicated. The Benton profile does not show any great variation in the A₁, A₂ and B₁ horizons, and in the case of Shouldice there is actually an accumulation in the A₂ horizon.

The high replaceable calcium content of the B₂ horizon of the various profiles is attributed to calcium carbonate. There is no corresponding increase of replaceable magnesium or sodium-potassium in this horizon in the black and the wooded profiles, but a more or less proportionate increase of magnesium is noticed in the brown soils.

The following conclusions are drawn from the preceding discussion :

(1) In the case of the wooded profiles the leaching processes have been more severe in the A horizons than in the black soils and have actually removed a part of the replaceable bases. There has also been a removal of the complex itself as the base-holding capacity is lower in these horizons than it is in the B_1 horizons of the wooded profiles or A horizons of the black soils.

(2) The bases that have moved in the black and brown soils were in the form of salts. There is no pronounced depletion of replaceable bases in the A_2 horizon indicated in these two soil groups. There is also very little difference in the base-holding capacity between the A_2 and B_1 horizons in these soils, so there has been no removal or disintegration of the complex. Although magnesium and sodium-potassium may have moved down with calcium as salts, their greater solubility prevented them from precipitating in the B_2 horizon.

(3) There has been less movement of water-soluble calcium and magnesium in the brown soils, as there is some carbonate found throughout the profile. The somewhat proportionate increase of magnesium in the B_2 horizon here indicates that the conditions have been too dry to carry it farther down even though it is more soluble.

All the profiles studied have replaceable calcium, magnesium and sodium-potassium in normal proportions. In a normal soil the replaceable bases are in the following proportions: calcium 75-90%, magnesium 10-25% and a small percentage of sodium-potassium. The Fort Vermilion profile shows a greater content of replaceable magnesium and sodium potassium than is found in the other profiles.

The soils of Alberta also compare very favorably with some of the other soils reported. Bray (2) worked with a number of Illinois soils and found the replaceable bases to be less than 33 M.E. per 100 gm. of soil. Page and Williams (11) determined the replaceable bases in the surface soil of the Broadbalk field at Rothamsted and found them to be between 12 and 16 M.E. In Alberta the replaceable base content of the A horizon is between 7.2 and 60 M.E.

In studying the degree of saturation of the various horizons it was found that the calcium chloride method did not give very consistent results. By this method most of the soils showed a base-holding capacity less than the amount of replaceable bases. This is probably due to removal of electrolytes by solution, and of complex by hydrolysis, in the course of leaching and washing. This method is not very satisfactory for demonstrating the small degree of unsaturation found in these soils. However, in samples in which the degree of unsaturation is more pronounced, this method is fairly satisfactory, as, for example, in the cases of the A_2 horizon at Fort Vermilion, the A_3 horizon at Ksituan and the B_1 horizon at Breton.

The most pronounced difference in the degree of saturation is noticed in the A_2 horizon. In the black soil this horizon is about 95% saturated in the Edmonton profile, and about 90% in the Hobbema. In the wooded profiles it is between 55% at Fort Vermilion and 88% at Wabamun. This is not a

high degree of unsaturation although the soil has a very leached appearance. This small degree of unsaturation accounts for the more or less neutral reaction of the wooded soils ($\text{pH} > 5$). From this it could be assumed that the aluminosilicate complex has been removed from the A_2 horizon of the wooded soils as a colloidal solution and not by disintegration into oxides of aluminium, silicon and some iron. This is considered to be the case, as the conditions are not very acid, so that very little disintegration of the complex should occur. However, it is probable that the complex enters a more dispersed state by being made more acidic. The accumulation of silicon dioxide in the A_2 horizon is attributed to disintegration of feldspar rocks into base-exchange complex and silica. Truog and Chucka (15) have found this to occur in weathering of feldspars.

The A_0 horizon of the wooded and the A_1 horizon of the black soils show about the same degree of both hydrolytic and replaceable acidity. There is considerably more hydrolytic acidity than replaceable acidity in this horizon, which shows that considerable hydrogen has been taken in. There is, however, no great acidity, the pH of this soil being above 6 as a rule.

The B_1 horizon of the black soils shows very little hydrolytic or replaceable acidity. On the other hand, the A_3 horizon from Ksituan and the B_1 horizon from Breton and Wabamun all show a fair degree of hydrolytic acidity. This presence of hydrolytic acidity in the A_3 and B_1 horizons of the wooded profiles is probably due to precipitation of colloids brought down in the course of leaching. These colloids have some hydrolytic acidity, probably slightly more than those remaining in the upper horizons. However, it is not necessary that all the hydrolytic acidity be neutralized in order to precipitate these colloids. This may account for the presence of this less active acidity in the A_3 and B_1 horizons. These colloids are organic and inorganic, the former being indicated by an increase in nitrogen content.

From the above it could be assumed that in case of the black soils, the A_2 and B_1 horizons have been subjected to less acid conditions than A_1 . Apparently as much hydrolytic acidity has been found in the A_1 horizons of the black soils as in the wooded, but it did not permeate the lower horizons to as great an extent.

The lack of hydrolytic acidity in the brown soils is accounted for by less leaching away of bases, and less production of organic acids.

A very important point is brought up by Gedroiz (3) with respect to leaching. He states that a complex will actually absorb H ions from pure water, with subsequent leaching away of bases, *i.e.*, hydrolysis of the complex will occur. Thus it may be possible that the acidity found in more humid regions is derived, not only from acid leaching, but also from excessive leaching.

It is seen from Table II that only the wooded soils show any indication of replaceable acidity. Thus, according to the Gedroiz system of classification, they fall into the podsol group. It also appears that the black soils are approaching a podsol condition as eventually acidity will pass from a hydrolytic to a replaceable stage.

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STUDIES ON HOMOGENEOUS FIRST ORDER GAS REACTIONS

III. THE DECOMPOSITION OF PARALDEHYDE¹

By C. C. COFFIN²

Abstract

The gaseous decomposition of paraldehyde to acetaldehyde has been studied from points of view already outlined. The reaction, which was followed by increase of pressure at constant volume, is homogeneous, accurately first order, and is presumably uncatalyzed. Its velocity has been measured between 209° and 270°C. at initial pressures of from 1.18 to 52.0 cm. of mercury. It goes to completion under these conditions of pressure and temperature at a rate which is independent of the total pressure and of the partial pressures of paraldehyde, acetaldehyde and mercury vapor. The activation energy is 44160 cal. per mol.

The velocity constants are given by the equation $\ln k = 34.83 - \frac{44160}{RT}$. The bearing of the data on the probably trimolecular reverse reaction as well as on work already reported is discussed.

Introduction

A systematic investigation of homogeneous first order gas reactions is being carried out in this laboratory (1, 2, 3). The main object of the work is to determine to what extent activation energies, as determined from temperature coefficients of reaction rate, are characteristic of molecular structure and how far they may be regarded as quantitative measures of bond stability. It appears also (3) that this work may lead to interesting information regarding the distribution of intramolecular energy. The present paper deals with the gaseous decomposition of paraldehyde to acetaldehyde—a reaction which has been found to go smoothly to completion at a measurable and reproducible velocity over a conveniently attained temperature range. The reaction is homogeneous and monomolecular. It is eminently suited for manometric measurements as the pressure increases 300%. It is of the first order and exhibits no change in velocity over the pressure range investigated, *viz.*, from total pressures of about 1 to 150 cm. of mercury. The reaction is being studied at lower pressures in an attempt to determine the number of squared terms involved in the activation process, and at higher pressures with a view to measuring the velocity of the probably trimolecular reverse reaction. The experiments are also being extended to include polymers of other aldehydes in order to obtain comparisons of the *E*'s and *A*'s of the Arrhenius equation (*cf.* 3). It is to be noted that a paraldehyde decomposition is essentially the change of three ethereal to three carbonyl oxygen molecules as against the one ethereal to one carbonyl oxygen shift of the previously reported ester decompositions.

Although some 120 experiments on the decomposition of paraldehyde have been made to date, only about 55 are concerned with the non-catalytic thermal reaction under conditions where the decomposition is complete, *i.e.*, at pressures

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low enough to allow ordinary manometric methods to be used. Only these latter runs are reported here as the measurement of equilibria and reaction velocities (catalyzed and non-catalyzed) at higher pressures still presents numerous difficulties.

Experimental

Apparatus and Technique

The first 21 runs were carried out in the apparatus already described (3). The remaining 34 were made in the apparatus represented in Fig. 1, which is self-explanatory. Xylene was boiled in *H* to prevent condensation of paraldehyde in the manometer when the mercury surface was held at *D*.

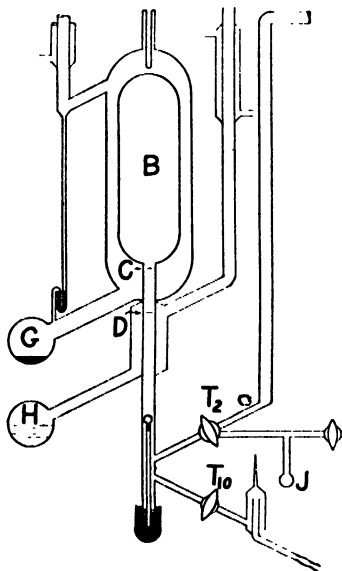


FIG. 1. Diagram of apparatus.

A large oil manometer was added beside *M*₃ (2, Fig. 1) for more accurate determination of the lower pressures on the boiling mercury in *G*.

Purification of the Paraldehyde

Two different samples of paraldehyde were used in these experiments. One was obtained by repeated fractional distillation of good commercial paraldehyde. The other was fractionated from c.p. paraldehyde which had been refluxed over, and distilled from, a large excess of metallic sodium. No difference in the decomposition velocities of the two samples was detected and it is therefore believed that no catalytic impurity was present in either.

The Product of the Reaction

As the pressure exactly tripled during each run no great pains were taken to obtain further proof that the product of the reaction was acetaldehyde. After several of the higher pressure runs however the mercury in *N* was lowered to *T*₁₀ and the reaction products were condensed through the three-way tap *T*₂ into the previously evacuated small bulb *J* by means of carbon dioxide and ether. The vapor pressure of the liquid in *J* when measured shortly after condensation was found to be identical with that of acetaldehyde in that it indicated a boiling point under atmospheric pressure of about 20°C. On standing, the vapor pressure invariably fell and the melting point rose, affording further evidence that the original liquid in the bulb was acetaldehyde which slowly polymerized to paraldehyde.

Results

As the reaction is of the type $A \rightarrow 3B$ the partial pressure of paraldehyde at time *t* is given by $\frac{1}{2}(3P_0 - P)$ where *P*₀ is the paraldehyde pressure at the

beginning and P is the total pressure at time t . The monomolecular velocity constant is therefore given by the equation

$$k = \frac{1}{t} 2.303 \log \frac{2P_0}{3P_0 - P}.$$

As before (2, 3), the expression $\log \frac{2P_0}{3P_0 - P}$ was plotted on a large scale against time and the velocity constant was determined for each run from the slope of the best straight line drawn through the points. For any one run all these points invariably fall on a perfectly straight line. For different runs, however, carried out under as nearly as possible identical conditions, the slope of these lines varied somewhat as may be seen from the constants of Tables I and II. This suggests that the reaction is sensitive to traces of some catalyst which is present in different amounts in different runs and which, as other experiments indicate, is introduced with the mercury.

In an attempt to identify this supposed catalyst a few runs were made in the presence of air, water vapor and stopcock grease. The velocity constants were usually slightly greater than those obtained in the absence of these substances although the observed acceleration was not in proportion to the amount of impurity added.

In practically all runs the observed final pressure agreed well within 1% with that calculated from the weight of paraldehyde taken, so that it is immaterial which value is used as $3P_0$. This is shown in Table I where both values are listed for a few typical runs at three different temperatures. Column 1 shows one-third of the observed final pressure, column 2 the value of P_0 calculated by the ideal gas laws from the weight of paraldehyde taken and column 3 the velocity constants. In the lower temperature runs where it was possible to obtain P_0 by a short extrapolation it was always found that the observed and calculated values agreed within the limits of error of the extrapolation.

That the specific reaction rate is independent of pressure is well shown in Table II which summarizes the data of the thermal decompositions at seven different temperatures. The initial pressures listed are one-third the observed final pressures and were used in calculating the accompanying constants. At no temperature do the constants show any definite drift as the pressure increases so that within this pressure range the

TABLE I
OBSERVED AND CALCULATED INITIAL PRESSURES*

Final press. 3	P_0 calc.	$k(\text{sec}^{-1})$
$T = 519.3^\circ\text{A.}$		
17.74	17.85	3.06×10^{-4}
24.60	24.88	2.96×10^{-4}
22.87	22.99	2.88×10^{-4}
$T = 526.8^\circ\text{A.}$		
14.12	14.22	5.41×10^{-4}
15.19	15.40	5.15×10^{-4}
8.92	9.00	5.31×10^{-4}
30.73	30.91	5.11×10^{-4}
10.94	10.96	5.52×10^{-4}
$T = 534.9^\circ\text{A.}$		
15.47	15.48	0.904×10^{-3}
22.87	23.04	1.00×10^{-3}
6.79	6.82	1.01×10^{-3}
19.69	19.78	0.920×10^{-3}
5.08	5.07	1.09×10^{-3}

*In cm. of mercury.

the constants show any definite drift as the pressure increases so that within this pressure range the

TABLE II
SUMMARY OF THE DATA OF THE THERMAL DECOMPOSITIONS

Temp.	P_0	$k \times 10^4$	Temp.	P_0	$k \times 10^4$	Temp.	P_0	$k \times 10^4$
482.1	12.94	1.11	519.3	1.36	3.06	534.9	3.07	1.09
	20.61	1.05		1.70	2.99		5.02	1.04
	24.96	1.23		1.80	3.34		5.08	1.09
	Mean	1.13		5.90	2.99		5.40	1.05
				8.06	3.14		6.79	1.01
501.9	5.37	6.31		10.17	3.06*		7.50	1.07
	14.56	6.15		11.24	3.04*		15.47	0.904
	16.80	6.29		13.57	3.00		15.83	1.09
	20.49	6.54		17.74	3.06		19.69	0.920
	20.51	6.59		20.95	3.00		22.87	1.00
	20.97	6.37		22.87	2.88		37.47	0.973
	Mean	6.34		24.60	2.96		Mean	1.02
		$k \times 10^4$		Mean	3.05			
512.2	10.25	1.66*				542.8	3.23	1.96
	14.84	1.66		1.18	5.65		3.76	1.95
	21.78	1.55	526.8	1.30	5.49		6.59	1.91
	25.76	1.63		2.42	5.82		7.10	1.94
	36.50	1.73		4.18	5.33		18.04	1.88
	38.94	1.56*		8.92	5.31		Mean	1.93
	52.00	1.50		9.67	5.44*			
	Mean	1.61		10.94	5.52			
				13.52	5.56			
				14.12	5.41			
				15.19	5.15			
				30.73	5.11			
				Mean	5.44			

reaction is strictly first order. The italicized constants in Table II were obtained in the presence of mercury vapor saturated at the temperature of

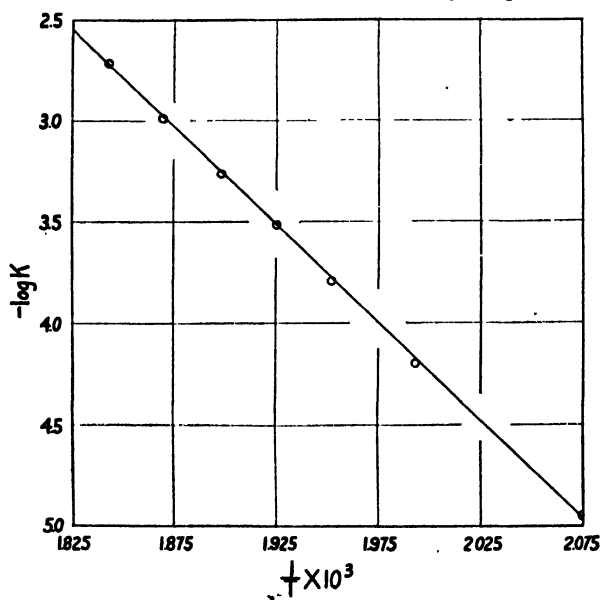


FIG. 2. Graph showing relation between $-\log k$ and $\frac{1}{T}$.

the reaction tube. The others were obtained in the presence of mercury vapor saturated at the temperature of boiling xylene, *i.e.*, at 1.7 mm. pressure. It is evident that mercury vapor between 0.17 and 10.00 ($T = 534.90^\circ$ A.) cm. pressure is without influence on the rate of decomposition.

Homogeneity of the Reaction

In Table II the values marked with an asterisk are the results of experiments in which the glass surface in contact with the paraldehyde vapor was enor-

mously increased by packing the reaction chamber with glass wool. It is evident that the extent of glass surface is without influence on the rate of the reaction which must therefore take place homogeneously throughout the gas.

While the final pressures of the decompositions in the unpacked bulb were found to remain constant to within 0.1 cm. for an indefinite time even at the highest temperatures, the final pressures of the runs in the packed bulb invariably decreased at a rate which was apparently dependent on the temperature, the total pressure and the amount of glass wool in the reaction chamber. A decrease of as much as 10 cm. in 24 hr. was observed at 519°A., with an initial aldehyde pressure of about 30 cm. After such an experiment the inside of the cold tubing leading to the reaction chamber was covered with yellow oily droplets of a high boiling liquid having an odor reminiscent of geraniol. The alkaline surface of the glass wool evidently brings about some sort of aldehyde condensation.

The Energy of Activation

In Fig. 2, $\log k$ is plotted against $1/T$. The temperatures and average constants of Table II were used. The slope of the straight line corresponds to an activation energy of 44,160 cal. per mol. Velocity constants are given by the equation $\ln k = 34.83 - \frac{44160}{RT}$.

Discussion

It is of interest to consider the reverse reaction in the light of the data already available. Roozeboom (9) and Hollmann (6) found the "natural" critical temperature of the system to be 218–221°C. and the equilibrium concentration of paraldehyde to be 11 mol. per cent. The reverse reaction thus takes place at a measurable rate within the temperature range over which the paraldehyde decomposition has been investigated, if the pressure is sufficiently high. Moreover, as the decomposition of paraldehyde is homogeneous the reverse reaction must likewise be homogeneous since a catalyst (e.g., a glass wall) cannot change an equilibrium. Unfortunately the critical pressures and absolute concentrations were not measured so that the specific velocity of the presumably third-order formation of paraldehyde cannot be estimated from that of its first order decomposition. Even if this were possible such an extrapolation of low pressure data would be anything but reliable as the velocity (8) and order (10) of a reaction may change considerably with pressure.

The activation energy (E_1) of a reaction and that (E_2) of its reverse are related to the heat of reaction Q by the equation $Q = E_1 - E_2$.

Cooper (4, 5) in this laboratory found the heat of the reaction, 3 acetaldehyde \rightarrow paraldehyde, to be 19,400 ($\pm 1\%$) cal. per mol. A less reliable value of something over 20,000 cal. is obtained from the heats of combustion (7). Taking Q as 19,400 and E_1 as 44,200, E_2 is found to be 24,800 cal. per mol of paraldehyde formed—an activation energy of the order of magnitude to be expected for a trimolecular reaction occurring under the above conditions of pressure and temperature.

As stated above further experiments along these lines are in progress.

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THE ELECTRIC MOMENT OF HYDROGEN PEROXIDE¹

BY E. P. LINTON² AND O. MAASS³

Abstract

The electric moment of hydrogen peroxide was found to be 2.13×10^{-18} in dioxan. Comparative measurements made on the electric moment of water gave 1.90×10^{-18} in dioxan. Measurements made with both water and hydrogen peroxide in ether gave lower values for their electric moments, and the reason for this is discussed. The structure of hydrogen peroxide is discussed, and the electric moment shown to be in agreement with all data as determined in this laboratory in indicating the presence of a co-ordinate co-valent bond.

The dielectric constant of a substance has always been considered an important physical property. In the last few years considerable advances have been made in the field of molecular structure due to the work of Debye (4, 5) and Smyth (11, 12), these investigators having made possible the calculation of the electric moment of a molecule from dielectric-constant data.

Theoretical

There are two general methods for finding the electric moment of the molecule. The first, due to Debye, consists of measuring the temperature coefficient of the dielectric constant of a gas, while the second consists of the measurement of the dielectric constant of dilute solutions of a substance in a non-polar solvent. The second method was used in this investigation as hydrogen peroxide is not stable in the gaseous state.

As the theory of dielectric polarization has been discussed elsewhere (4, 5, 11, 12) only the equations immediately necessary will be given here. The molar polarization of a substance in which the molecules are free to assume a random orientation, as in the gaseous state, is given by

$$P = \frac{\epsilon - 1}{\epsilon + 2} \frac{M}{d} = \frac{4\pi}{3} \gamma N + \frac{4\pi N}{d} \frac{\mu^2}{T}, \quad (1)$$

where ϵ = dielectric constant, M = molecular weight, d = density, N = number of molecules in a gram molecule, γ = molecular polarizability, κ = molecular gas constant, T = absolute temperature, and μ = electric moment of a molecule.

The term $\frac{4\pi}{3} N\gamma$ is the polarization due to shifts induced in the molecule by the external field, and is usually calculated as the molar refraction of light at infinite wave-length. The expression $\frac{4\pi}{3} N\gamma$ includes both the electronic shifts induced and the shifts induced in the atom or radicals. Therefore $\frac{4\pi}{3} N\gamma = P_E + P_A$.

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The expression $\frac{4\pi}{9\kappa} \frac{\mu^2}{T}$ gives the moments of the molecules oriented by the electric field equal to P_M . The total polarization is then, $P = P_E + P_A + P_M$.

The above equations apply to pure substances in the gaseous state. In liquids, however, the molecules are so close together that if they contain doublets they affect one another so that their freedom of motion is influenced. If the molecules of a polar liquid are separated from one another by molecules of a non-polar liquid, the polar molecules should behave much as in the gaseous condition. The molar polarization of the mixture of the two liquids is given by

$$P_{12} = \frac{\epsilon - 1}{\epsilon + 2} \frac{C_1 M_1 + C_2 M_2}{d} = C_1 P_1 + C_2 P_2, \quad (2)$$

where C_1 and C_2 , M_1 and M_2 , P_1 and P_2 are the molar fractions, molecular weights and polarizations of the two components, and

$$C_1 = 1 - C_2,$$

$$P_2 = \frac{P_{12} - P_1}{C_2} + P_1. \quad (3)$$

Thus the polarizations of the polar liquid are calculated at various concentrations. These polarizations are plotted against mole fraction and the value of P_2 at infinite dilution (*i.e.*, P_{00}) is obtained.

$$P_{00} = P_E + P_A + P_M \quad \frac{P_M = P_{00} - MR_{00}}{P_{00} - MR_{00} T},$$

$$= 0.0127 \times 10^{-18} \sqrt{P_{00} - MR_{00} T},$$

neglecting P_A which is very small for water or hydrogen peroxide.

In the experiments carried out by the authors, ether and dioxan were used as the non-polar solvents. Ether has a higher dielectric constant than the ordinary non-polar solvents and it is possible that hydrogen peroxide would behave quite differently in ether than in an ordinary non-polar solvent such as dioxan. Dioxan has been used as a solvent by Williams (15) and Smyth (14). They considered that it compared favorably with benzene or hexane as a solvent for the determination of electric moments. Dioxan is miscible with water and hydrogen peroxide in all proportions and dissolves many substances which are insoluble in the ordinary non-polar liquids. The electric moment of water has been determined by several investigators (7, 9, 15, 16) both in the liquid and gaseous state. In order to make certain that the method gave correct results the experiments were carried out on dilute solutions of water in ether and dioxan. In this way it was possible to compare the results for both water and hydrogen peroxide in the two solvents.

Apparatus

The resonance method for the measurement of the dielectric constant has been described in detail (8). A power tube was used in the variable oscillator to overcome any conductivity effects in the solutions. The dielectric cell was made of pure block tin and was described previously (8).

In order to avoid as much as possible the loss of ether by volatilization the dielectric-constant measurements were carried out at 10°C. in the case of the ether solutions. The solutions were made up by adding a weighed quantity

of hydrogen peroxide or water to a known quantity of ether or dioxan. Additional amounts of hydrogen peroxide and water were added in order to give solutions of increasing concentration. Smyth's value (14) of 2.306 was taken as the dielectric constant of pure dioxan at 25°C. The dielectric constant of pure ether at 10°C. was taken to be 4.52.

Purification of Materials

The dioxan was obtained from the Carbide and Carbon Chemicals Corporation. It was recrystallized twice and distilled from sodium: boiling point, 101.3°C. at 760 mm. pressure; density, 1.031 at 25°C; refractive index, 1.4203; dielectric constant, 2.306.

The ether was shaken with its own volume of water five times, dried over calcium chloride and distilled from sodium: density, 0.726; dielectric constant, 4.52 at 10°C.

Hydrogen peroxide was prepared in the usual way by distillation and concentration of the 30% crude material. The hydrogen peroxide (96%) was recrystallized twice to give peroxide approximately 99% pure. This sample was used in making up the solution by adding it from a weight pipette.

The ordinary distilled water of the laboratory was used to make up the dilute solutions of water.

Results

Table I shows the experimentally determined dielectric constants and the density of the liquid mixtures, together with the values of the polarization P_{12}

TABLE I
DIELECTRIC CONSTANTS, DENSITIES AND POLARIZATIONS

Mole fraction water	Dielectric constant	Density	Polarization		Mole fraction water	Dielectric constant	Density	Polarization	
			P_{12}	P_2				P_{12}	P_2
Water in dioxan, 25° C.					Hydrogen peroxide in ether, 10° C.				
0.000	2.306	1.0311	25.85	25.85	0.0000	4.52	0.726	55.0	55.0
0.043	2.542	1.0310	28.05	78.00	0.0312	4.97	.735	56.35	98.3
0.078	2.790	1.0300	29.85	77.50	0.0512	5.32	.742	57.25	99.0
0.114	3.120	1.0290	32.15	79.60					
Water in ether, 10° C.					Hydrogen peroxide in ether, 0° C.				
0.0000	4.52	0.726	54.95	P_1	0.1022	6.6	0.769	59.3	99.8
0.0282	4.74	.727	55.35	69.8	0.1950	8.7	.791	60.2	82.5
0.0430	5.07	.729	55.80	73.6	0.3230	13.2	.834	58.8	67.2
					0.4360	18.0	.892	53.8	52.8
					0.5740	26.9	.954	48.2	43.3
					0.6540	33.8	.997	44.0	38.3
					0.7500	44.3	1.071	38.2	32.7
					1.0000	91.0	1.460	22.6	22.6
Hydrogen peroxide in dioxan, 25° C.									
0.00000	2.306	1.031	25.85	25.85					
0.02104	2.451	1.034	27.30	94.2					
0.04105	2.570	1.036	28.50	89.4					
0.06100	2.722	1.039	29.70	88.2					

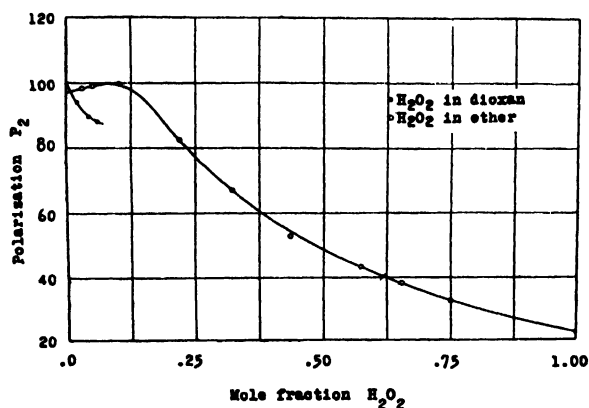


FIG. 1. Relation between polarization of hydrogen peroxide and mole fraction in solvent.

TABLE II
MOLAR REFRACTION, POLARIZATION AT INFINITE DILUTION AND MOMENT
OF WATER AND HYDROGEN PEROXIDE

	MR_D	P_{∞}	$P_{\infty} - MR_D$	$\mu \times 10^{18}$
Water in dioxan	3.7	79.1	75.4	1.90
Water in ether	3.7	67.2	63.5	1.71
Hydrogen peroxide in dioxan	5.6	100.2	94.6	2.13
Hydrogen peroxide in ether	5.6	97.7	92.1	2.06

Discussion

The values given for water and hydrogen peroxide neglect the atomic polarization P_2 . This term may be disregarded when the dielectric constant of the liquid is large and its molecule contains only a small number of atoms. The value for the moment of water in dioxan agrees very closely with the values of other investigations (7, 9, 15, 16).

The value found for water in ether is considerably lower than the value found in other solvents. The measurements in ether solution were not as accurate as the measurements in the other experiments due to the small solubility of water in ether, making the extrapolation of P_2 to infinite dilution uncertain. The difference in the values of the electric moment of water may be due to the fact that the measurements in ether were carried out at a lower temperature in order to avoid the loss of ether by volatilization. The value found for the temperature coefficient of the dielectric constant of water shows that the dielectric constant increases rapidly at temperatures below 15°C. (6). This large increase is probably due to a change in the association of water at these temperatures. Thus, at 10°C., the temperature at which these determinations of the electric moment of water in ether were carried out, the value found for the electric moment of water may be considerably lower than the value found at 25°C.

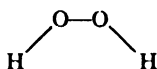
calculated by means of Equation (2). P_2 is calculated from P_{12} as shown in Equation (3).

The values for P_{∞} are obtained by extrapolation of the values of P_2 to infinite dilution. These extrapolations for hydrogen peroxide are shown in Fig. 1.

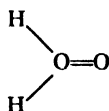
Table II shows the molar refraction, polarization at infinite dilution and moment of water and hydrogen peroxide.

On the other hand, the higher dielectric constant of ether should cause the water to be less associated than it is in dioxan solution (13). Evidently the temperature effect is considerably larger than the effect due to the large dielectric constant of the ether. As was to be expected, the electric moment of hydrogen peroxide is greater than the moment of water. As in the case of water, the moment in the ether solution is less than in the dioxan solution, probably for the same reasons.

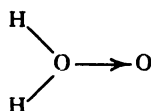
The high electric moment of water has been explained by assuming an unsymmetrical arrangement for the atoms in the molecule. Debye (8) has given a detailed mathematical analysis of the subject, and concludes that the molecule which best fits the facts is that in which the valence bonds are at an angle of 64° with one another. Hydrogen peroxide may have any of the following formulas:—



I



II



III

All physico-chemical measurements, such as parachor (1), molecular refractive power (3), and high dielectric constant (2), indicate that III is the correct form. The ease with which hydrogen peroxide loses an oxygen atom and forms water is in agreement with this.

The high electric moment of hydrogen peroxide is against Formula I, as this formula would have a very small moment in comparison with the others.

Formula III contains a co-ordinate co-valent bond which usually gives molecules large electric moments (10). Therefore the high electric moment of hydrogen peroxide points to Formula III as being the correct one. To sum up, all data as determined in this laboratory are in favor of this structure.

The calculation of an angle between valence bonds is left for the present.

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PROPAGATION OF ULTRASOUND IN SOLID CYLINDERS (TRANSVERSE WAVES)¹

By R. RUEDY²

Abstract

The expression giving the phase velocity c with which flexural waves pass through long solid rods is deduced for frequencies varying between zero and over 1,000,000 cycles per sec. and rods of any diameter. As the frequency increases, the velocity c increases gradually from very low values toward $c^2 = mE/2s(m+1)$, reached when the wave-length is much smaller than the diameter of the rod. Published experimental results for transverse waves are in good agreement with the theory given. In general at least four effects enter into the propagation of ultrasound through solid cylinders: first, longitudinal waves, for which the phase velocity decreases toward c as the frequency increases; second, transverse waves, for which the phase velocity increases toward c as the frequency increases; third, pure radial waves at certain frequencies; fourth, resonance effects between the different types of waves, which, on account of the mechanical coupling existing between them, change the natural period of vibration of the rod without affecting the velocity.

Introduction

When attempts are made to send purely longitudinal waves of high frequency through a solid rod, other types of motions besides those originally excited invariably appear (1-3, 6-8). That the solid particles set into vibration along the axis show at the same time motions perpendicular to this direction, has of course its reason in the contractions and expansions which a solid rod undergoes when transmitting longitudinal waves, the changes in thickness being given by Poisson's ratio. There is at no moment in the rod a cross-section in which the particles move only parallel to the axis, and the amplitude of the radial displacement increases from the interior toward the surface. On the other hand, the longitudinal and radial oscillations of the particles may be considered as components in part of a more general type of wave, the transverse or flexural kind, and experience has shown that over a certain range of frequencies flexural waves are very strongly excited, especially in cylindrical rods the diameter of which does not exceed a few millimetres. Both waves may be present at the same time and, in rods of finite length, produce their own set of stationary dust figures. It may become impossible to say to which wave a given figure belongs unless it is known in what way the velocity c of the wave changes with frequency.

The very high frequencies at which confusion is most likely to occur are of great practical importance as they belong to the range of broadcasting waves. Good stations maintain their carrier frequencies within very narrow limits, in some cases less than ten cycles, by means of oscillating rods or disks. It is, therefore, desirable to know the velocity of propagation of longitudinal and flexural waves from zero frequency to well over 1,000,000 cycles per sec., to ascertain whether the presence of both longitudinal and transverse waves leads to the formation of wave-groups having a higher velocity of propagation

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than the waves themselves, and to study the possible effects which the second wave-system might have on the natural frequencies of vibration of a rod of standard size in which longitudinal waves are excited for calibration purposes. Means for suppressing a certain type of wave are also of interest.

The Velocity of Transverse or Flexural Waves

When a flexural wave is sent through a cylindrical rod, the general equations of the motion of the particles are, in cylindrical co-ordinates r, θ, z ,

$$u = U(r) \cos \Theta e^{i(\gamma z + p t)} \quad v = V(r) \sin \Theta e^{i(\gamma z + p t)} \quad w = W(r) \cos \Theta e^{i(\gamma z + p t)},$$

u being the displacement in the direction of the radius, v that along θ , or the tangential displacement, and w the displacement parallel to the long axis z . The frequency f appears in $p = 2\pi f$ and the wave-length in $\gamma = 2\pi f/c$. The equations of motion furnished by the theory of elasticity lead to the following solution (4, §. 214),

$$U(r) = A \frac{\partial J_1(kr)}{\partial r} + B \gamma \frac{\partial J_1(kr)}{\partial r} + C \frac{J_1(kr)}{r}$$

$$V(r) = -A \frac{J_1(kr)}{r} - B \gamma \frac{J_1(kr)}{r} - C \frac{\partial J_1(kr)}{\partial r}$$

$$W(r) = A i \gamma J_1(kr) - B i k^2 J_1(kr)$$

with

$$h^2 = \frac{p^2}{c_0^2} \frac{(m+1)(m-2)}{m(m-1)} - \frac{p^2}{c^2} = \frac{p^2}{c^2} \left(\frac{c^2}{c_0^2} \frac{(m+1)(m-2)}{m(m-1)} - 1 \right)$$

$$k^2 = \frac{p^2}{c_0^2} \frac{2(m+1)}{m} - \frac{p^2}{c^2} = \frac{p^2}{c^2} \left(\frac{c^2}{c_0^2} \frac{2(m+1)}{m} - 1 \right),$$

$E/s = c_0^2$, the square of the velocity of longitudinal waves of low frequency, and $2 < m < 5$, where m designates the reciprocal of Poisson's ratio. J_1 is the Bessel function of the first kind of order one, and as it becomes equal to zero when the argument is zero, points lying on the central axis evidently do not move along the axis, but only in a plane perpendicular to it, in contrast with the conditions found for a longitudinal wave. Moreover because the traction must vanish at the surface the following equations must be satisfied over the entire length of the cylinder, wherever $r = a$, the radius of the cylinder:

$$\frac{u}{a} + (m-1) \frac{\partial u}{\partial a} + \frac{1}{a} \frac{\partial v}{\partial \theta} + \frac{\partial w}{\partial z} = 0$$

$$\frac{v}{a} - \frac{1}{a} \frac{\partial u}{\partial \theta} - \frac{\partial v}{\partial a} = 0$$

$$\frac{\partial u}{\partial z} + \frac{\partial w}{\partial a} = 0$$

or

$$i \gamma U(a) + \frac{\partial W}{\partial a} = 0$$

$$\frac{U}{a} + \frac{V}{a} - \frac{\partial V}{\partial a} = 0$$

$$\frac{U}{a} + \frac{V}{a} + (m-1) \frac{\partial U}{\partial a} + i \gamma W = 0$$

These boundary conditions yield therefore three equations from which A , B and C may be eliminated, and the phase velocity $c = p/\gamma$ calculated in terms of

the radius a and the frequency f . After the derivation has been carried out the three boundary conditions become

$$\begin{aligned} 2hJ_2(ha)A + 2\gamma kJ_2(ka)B - (2kJ_2(ka) - k^2aJ_1(ka))C &= 0 \\ 2\gamma aJ_1'(ha)A + (\gamma^2 - k^2)aJ_1'(ha)B + \gamma J_1(ka)C &= 0 \\ A[(m-2)(hJ_2(ha) - h^2aJ_1(ha)) - (\gamma^2 + h^2)aJ_1(ha) \\ &\quad + B\gamma(m-2)(kJ_2(ka) - k^2aJ_1(ka)) \\ &\quad - (m-2)kJ_2(ka)C = 0, \end{aligned}$$

and the final result is obtained in the following form:

$$\begin{aligned} &2ka \frac{J_2'(ka)}{J_1'(ka)} \left[(\gamma^2 - k^2) \left(h^2 + \frac{\gamma^2 + h^2}{m-2} \right) \right] \\ &+ \frac{J_2(ka)}{J_1(ka)} \left[6\gamma^2 k^2 - \left(h^2 + \frac{\gamma^2 + h^2}{m-2} \right) (2\gamma^2 + (\gamma^2 - k^2)(2 + k^2a^2)) \right] \\ &+ h k \frac{J_2(ha)}{J_1(ha)} \left[(\gamma^2 + k^2 + 2\gamma^2 k^2 a^2) - ka \frac{J_2(ka)}{J_1(ka)} (6\gamma^2 - (\gamma^2 - k^2)) \right] \\ &- ka \left[2\gamma^2 k^2 - \left(h^2 + \frac{\gamma^2 + h^2}{m-2} \right) (\gamma^2 - k^2) \right]. \end{aligned}$$

When for a given frequency f , a certain phase velocity c is assumed, the values γ , k , $J_2(ka)/J_1(ka)$, h , $J_2(ha)/J_1(ha)$ follow immediately and on introducing them into the equation, the correctness of the choice of c can be tested from case to case. Three trials suffice in general for extrapolating to the correct value. For preparing charts showing, for a certain material, how the velocity of propagation of the transverse waves varies with frequency and diameter of the rod, the simplest procedure is to assume a series of values of c for the same frequency and to make these values fit the equation by the proper choice of a .

Discussion of the Velocity Formula

On account of the nature of the Bessel functions J_2 and J_1 a discussion of the general solution would be difficult and not necessarily useful; moreover quite different velocities of propagation would likely be found for the same frequency. For the physical problem at hand, account must be taken of the fact that for thin rods and low frequencies both experiment and theory show that c is given by the equation

$$c^2 = \pi a c_0 f,$$

that is, it varies with frequency according to a square law, being very much smaller than c_0 , the velocity of longitudinal waves at low frequencies. Only those solutions of the general equation will therefore be retained which go over into the values known to be valid for ordinary frequencies, and discontinuous changes of the velocity will be excluded unless they are to be expected in a certain range on physical grounds. This means that starting with frequencies in the neighborhood of zero, both h and k are imaginary quantities. (The letters h and k will, however, be retained to designate the real part as if what had been called h and k up to this point had actually been $h' = ih$ and $k' = ik$.) Replacing at the same time part of the symbols h and k by their values, the general solution thus becomes

$$\begin{aligned}
& ka \left(\frac{c^2}{c_0^2} \frac{m+1}{m} \right)^2 + 2ka \frac{J_2(ika)}{J_1(ika)} \left(\frac{c^2}{c_0^2} \frac{m+1}{m} - 1 \right)^2 \\
& + \frac{J_2(ika)}{iJ_1(ika)} \left[2 - 5 \frac{m+1}{m} \frac{c^2}{c_0^2} - \left(\frac{c^2}{c_0^2} \frac{m+1}{m} - 1 \right)^2 (2 - k^2 a^2) \right] \\
& + \frac{hk}{\gamma^2} \frac{J_2(ika)}{iJ_1(ika)} \left[\frac{m+1}{m} \frac{c^2}{c_0^2} - k^2 a^2 + \left(2 + \frac{c^2}{c_0^2} \frac{m+1}{m} \right) \frac{kaJ_2(ika)}{iJ_1(ika)} \right] = 0,
\end{aligned}$$

where h^2 and k^2 are positive quantities both smaller than γ^2 and $k < h$. As the ratio $\frac{J_2(ika)}{iJ_1(ika)}$ has only positive values (Table I) all the terms appearing in the equation become real.

TABLE I
VALUES OF $\frac{J_2(ig)}{iJ_1(ig)}$

g	$\frac{J_2(ig)}{iJ_1(ig)}$	g	$\frac{J_2(ig)}{iJ_1(ig)}$	g	$\frac{J_2(ig)}{iJ_1(ig)}$	g	$\frac{J_2(ig)}{iJ_1(ig)}$	g	$\frac{J_2(ig)}{iJ_1(ig)}$
0.0	0.00	1.0	0.240	2.0	0.433	3.0	0.568	4.0	0.658
0.2	0.05	1.2	0.283	2.2	0.464	3.2	0.589	5.0	0.719
0.4	0.099	1.4	0.324	2.4	0.494	3.4	0.608	10.0	0.854
0.6	0.148	1.6	0.363	2.6	0.520	3.6	0.626	15.0	0.902
0.8	0.195	1.8	0.399	2.8	0.545	3.8	0.642		

An insight into the way in which the velocity c varies with frequency may then be readily obtained by choosing simple fractions of $m/(m+1)$ as values for c^2/c_0^2 , such as $m/18(m+1)$, $m/8(m+1)$ up to $m/2(m+1)$, the largest value for which k remains positive and real. The following expressions correspond then to one another:

$$\begin{aligned}
& \frac{c^2}{c_0^2} \quad \frac{c^2}{c_0^2} \frac{m+1}{m} \quad h^2 \quad k^2 \quad \frac{hk}{\gamma^2} \\
& \frac{m}{x(m-1)} \quad - \frac{1}{x} \quad \gamma^2 \left(1 - \frac{m-2}{x(m-1)} \right) \quad \gamma^2 \left(1 - \frac{2}{x} \right) \quad \frac{1}{x} \sqrt{(x-2) \left(x-1 + \frac{1}{m-1} \right)}.
\end{aligned}$$

When c/c_0 increases both h and k fall, k more quickly than h , from the value unity for very low velocities toward zero. Writing y for $\gamma a = 2\pi fa/c$, the general equation now reduces to:

$$\begin{aligned}
& \frac{\sqrt{1-\frac{2}{x}}}{x^2} y + 2 \left(1 - \frac{1}{x} \right)^2 \sqrt{1-\frac{2}{x}} y \frac{J_2}{J_1^2} \left(i \sqrt{1-\frac{2}{x}} y \right) \\
& + \frac{J_2}{iJ_1} \left(i \sqrt{1-\frac{2}{x}} y \right) \left[y^2 \left(1 - \frac{2}{x} \right) \left(1 - \frac{1}{x} \right)^2 - \frac{1}{x} - \frac{2}{x^2} \right] \\
& + \frac{hk}{\gamma^2} \frac{J_2}{iJ_1} \left(i \sqrt{1-\frac{m-2}{x(m-1)}} y \right) \left[\left(2 + \frac{1}{x} \right) \sqrt{1-\frac{2}{x}} y \frac{J_2}{iJ_1} \left(i \sqrt{1-\frac{2}{x}} y \right) + \frac{1}{x} - \left(1 - \frac{2}{x} \right) y^2 \right] \\
& = 0,
\end{aligned}$$

where $\frac{J_2}{iJ_1}(iy)$ means $\frac{J_2(iy)}{iJ_1(iy)}$.

For a very low velocity or a very large x the equation becomes

$$\frac{J_2(iy)}{iJ_1(iy)} = \frac{y}{2},$$

with the solution $y=0=2\pi fa/c$, that is with the lowering of the velocities the frequencies tend even more rapidly toward zero than the velocity. Lessening the thickness of the rod has the same effect. On going to larger values of c and y , the coefficient h remains near unity much longer than k , and even for $x=4$ it has dropped to only 0.94 for $m=3$, and to 0.91 for $m=4$. For different values of m the equations differ from one another only by their last term, which is for $m=3$ (*i.e.*, for tin, or drawn aluminium of 92% purity, or drawn copper with about 0.2% As, as against $m=2.9$ for the pure metals)

$$\frac{\sqrt{(x-2)(x-0.5)}}{x} \frac{J_2}{iJ_1} \left(iy \sqrt{1 - \frac{0.5}{x}} \right) \left[\dots \dots \right],$$

and for $m=4$ (certain types of special glasses)

$$\frac{\sqrt{(x-2)(x-0.67)}}{x} \frac{J_2}{iJ_1} \left(iy \sqrt{1 - \frac{0.67}{x}} \right) \left[\dots \dots \right].$$

In Table II are shown the solutions y for various values of x , and $m=3$, and in Fig. 1 the computed points have been indicated on the experimental curves for aluminium for which approximately $m=3$.

TABLE II
SOLUTION OF THE PHASE VELOCITY EQUATION FOR $m=3$

x	18	8	4	3
c/c_0	0.204	0.307	0.433	0.500
$y=2\pi af/c$	0.5	0.75	1.35	1.86

For $x=2$ the equation becomes equal to zero identically, *i.e.*, for any frequency the velocity is $(m/2(m+1))^{1/2}$, corresponding to the case in which the wave-length of the purely transverse waves is much smaller than the diameter of the rod. Furthermore for $x=1$, we have

$$y-3 \frac{J_2}{J_1}(y) + \frac{1}{\sqrt{m-1}} \left(1+y^2-3y \frac{J_2}{J_1}(y) \right) \frac{J_2}{iJ_1} \left(iy \sqrt{1 - \frac{m-2}{m-1}} \right),$$

or, for $m=3$

$$y-3 \frac{J_2}{J_1}(y) + 0.707 \left(1+y^2-3y \frac{J_2}{J_1}(y) \right) \frac{J_2}{iJ_1}(0.707iy) = 0.$$

The question here is whether when k , which has become zero for $c^2=c_0^2m/2(m+1)$, passes over to negative values the velocity resulting from the equation continues to change gradually, in other words whether the equation for $x=1$ has a solution for rather small values of y . When y varies from 0 to 3.8317, the ratio $J_2(y)/J_1(y)$ varies from zero to infinity and beyond this point jumps suddenly to large negative values. The equation admits of a solution in the neighborhood of y equals two, that means for finite frequencies. Apparently even if the values of y are restricted to those smaller than 3.83 two different velocities result for each frequency. There is, however, a sudden change in

the type of motion at the point where k has dropped to zero so that it is uncertain whether the higher speeds have any physical significance.

As in the case of the elementary formula $c^2 = \pi a c_0 f$, valid for very thin rods and low frequencies, the velocity at higher frequencies and in thicker rods depends on the product af only, so that theoretically when the velocity c has been found for a certain frequency f and radius a , the same solution ought to apply to a higher frequency in a correspondingly smaller rod.

Motion of Particles

The equation governing the velocity of the transverse waves in a solid cylinder involves the assumption that the motion u along the radius of a particle lying at the distance r from the central axis; v , its motion perpendicular to u , but in the same cross-section; and w , the motion of the point parallel to the axis, are given by the following solution of the general theory:

$$u = \left[A \left(i h J_0(ihr) - \frac{J_1(ihr)}{r} \right) + B \gamma \left(i k J_0(ikr) - \frac{J_1(ikr)}{r} \right) + C \frac{J_1(ikr)}{r} \right] \cos \Theta e^{i(\gamma z + pt)}$$

$$v = \left[A \frac{J_1(ihr)}{r} - B \gamma \frac{J_1(ikr)}{r} - C \left(i k J_0(ikr) - \frac{J_1(ikr)}{r} \right) \right] \sin \Theta e^{i(\gamma z + pt)}$$

$$w = \left[A \gamma i J_1(ihr) + B k^2 i J_1(ikr) \right] \cos \Theta e^{i(\gamma z + pt)}.$$

As $i J_0(ihr)$ and $J_1(ihr)$ are positive and imaginary, and the constants A , B , C real quantities, the exponential $i(\gamma z + pt)$ term furnishes the function sine $(\gamma z + pt)$ for real values of u and w , and $\cos(\gamma z + pt)$ for v :

$$u = \left[A \left(\frac{J_1(ihr)}{ir} - h J_0(ihr) \right) + B \gamma \left(\frac{J_1(ikr)}{ir} - k J_0(ikr) \right) - C \frac{J_1(ikr)}{ir} \right] \cos \Theta \sin(\gamma z + pt)$$

$$v = \left[-A \frac{J_1(ihr)}{ir} + B \gamma \frac{J_1(ikr)}{ir} + C \left(k J_0(ikr) - \frac{J_1(ikr)}{ir} \right) \right] \sin \Theta \sin(\gamma z + pt)$$

$$w = \left[A \gamma i J_1(ihr) - B k^2 i J_1(ikr) \right] \cos \Theta \cos(\gamma z + pt).$$

As an example, the elongations in the direction parallel to the length of the

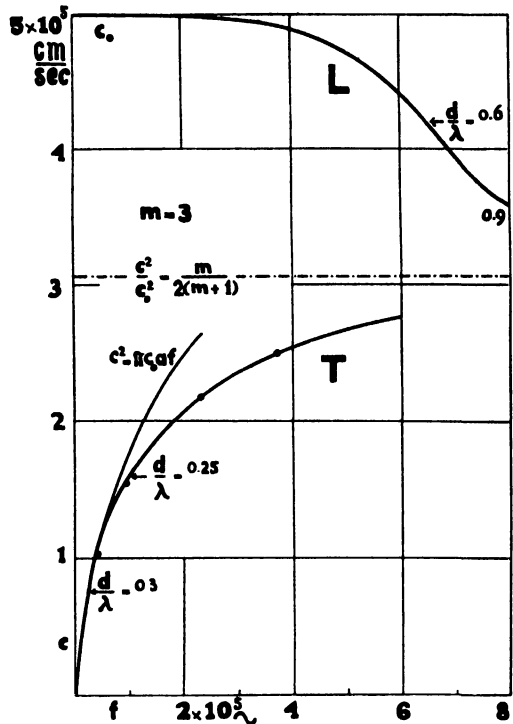


FIG. 1. Velocity of longitudinal (curve L) and transverse waves (curve T with computed points marked) in an aluminium rod 0.4 cm. thick and 35.1 cm. long (δ).

rod of the particles lying upon the surface have been computed for an aluminium rod 4 mm. thick (Table III).

TABLE III
VIBRATION w AT THE SURFACE OF AN ALUMINIUM ROD 4 MM. THICK

f per sec.	w
5×10^4	$(-0.69A + 0.199B) \cos \theta \sin (\gamma z + pt)$
10^5	$(-1.56A + 0.541B) \sin \theta \sin (\gamma z + pt)$
3.75×10^5	$(-11.83A + 5.62B) \cos \theta \cos (\gamma z + pt)$

When the experimental conditions are such that for the lowest frequency the greatest elongation is equal to one-half ($A \approx 1$, $B \approx 1$), this value will rapidly increase as higher frequencies are excited in the rod. It is also possible to imagine a case in which the transverse component w vanishes as the number of cycles increases, for instance when $A \approx 1$, $B \approx 2$. All this agrees with the observed fact that flexural waves are relatively strong over a certain range of the higher frequencies.

It is also of interest to compare the way in which the amplitude varies over the cross section in the case of longitudinal as against flexural waves. A particle set into motion by a longitudinal wave vibrates in the direction z according to the equation:

$$w = [-A\gamma J_0(ihr) - Ck J_0(kr)] \sin(\gamma z + pt).$$

Taking again as an illustration an aluminium bar of 6 mm. thickness, it is found that the amplitude of the longitudinal waves scarcely varies along the radius provided that the frequency does not exceed about 150,000 cycles. At 100 kc. for instance the ratio between edge and centre is about 0.98. This means that when one end of the rod is brought into close contact with an oscillating piston, such as is represented by an oscillating quartz plate, the end can adapt itself to the boundary conditions thus imposed without any strains building up over the cross section. At higher frequencies, however, there is a marked drop in the amplitude of w along the radius in the natural state of vibration, and the vibrations forced upon the end cause internal forces of deformation over the cross section, stresses which are relieved by the setting up of flexural and possibly still other forms of vibrations, such as surface waves for instance. The uneven distribution of w will tend to occur at lower frequencies for thicker rods as shown by theory and experiment (2, 6). One method of lessening the strength of transverse waves consists therefore in using as a source a vibrating disk in which the amplitude decreases from the centre toward the edge. Unfortunately a separate disk would have to be used for different ranges of wave-lengths. A thinner rod placed between the source and brought into contact with a thicker rod is likely to serve the same purpose over a limited band of frequencies.

It is by no means certain that all the points of the quartz disks as usually cut and employed for exciting vibrations in a rod move in phase, and when as is sometimes the case (5), a small layer of oil lies between disk and rod, the uneven

distribution of the forces in the sound field facing an oscillating piston surface is likely to complicate matters. However that may be, both transverse and longitudinal waves are as a rule directly set up in the rod and not the flexural waves by way of the forced longitudinal waves. To a certain extent this also applies to the radial waves, so that when a high frequency sound wave acts upon a rod it influences a system which would possess in the uncoupled state three distinct series of natural frequencies of vibration. On account of the unavoidable mechanical coupling existing between the different types of vibration, each natural period of vibration is influenced by the presence of one at least of the other types, so that two periods of resonance exist in each system, one higher and the other lower than the period of the uninfluenced systems. In the place of complete resonance, beats may appear between the two types of vibration (2). The actual effects to be expected depend, however, on the degree of damping which assumes different values in each case, particularly at high frequencies, where moreover the value of E is no longer a constant.

When solutions of the equation of motion are admitted in which the square of the velocity exceeds the value $c_0^2 m/2(m+1)$ so that ik becomes real, the type of motion changes completely; instead of a single cosine wave for w , for instance, there will be two waves:

$$w = A \gamma i J_1(ihr) \cos \Theta \cos (\gamma z + pt) - B k^2 J_1(kr) \cos \Theta \sin (\gamma z + pt).$$

There is as yet no experimental evidence of such a sudden change.

Possible Group Velocities

As it seems to be very difficult to prevent the appearance of flexural waves when high frequency sound waves act upon solid bodies, and as both types cause similar longitudinal and radial motions w and u at the surface of the rod, it is possible that the two systems form trains of waves, and that the velocity of the wave-groups is higher than the phase velocities. It is known that when two slightly different frequencies are exciting transverse waves in a bar, they will form a train of waves of the equation (5, p. 301)

$$\begin{aligned} y &= \cos (pt - \gamma z) + \cos (p't - \gamma'z) \\ &= 2 \cos \pi \left\{ t(f - f') - z(\gamma/2 - \gamma'/2) \right\} \cos \pi \left\{ t(f + f') - z(\gamma/2 + \gamma'/2) \right\}, \end{aligned}$$

and when the difference between the two frequencies is small, the group velocity will be about twice that of the phase velocity. In the case of two waves being sent into the bar from both ends, the two oscillators may not entirely be in resonance, on account of the coupling produced by the rod, and velocities of transverse waves even higher than those of any longitudinal wave are then possible. With purely longitudinal waves, or with transverse waves of very high frequency, for which according to the general formula the velocity changes only slowly with frequency, such an effect does not appear. It is also absent in the case in which two waves of the same frequency, but slightly different velocity, travel in the same direction.

The velocity of the radial waves and their interaction with the flexural and longitudinal waves will be discussed in a subsequent article.

Acknowledgment

The author is indebted to Dr. R. W. Boyle for his continued interest in this problem.

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MECHANICAL VIBRATIONS IN TRANSMISSION LINES¹

By C. D. NIVEN²

Abstract

Various types of vibration are discussed with special emphasis on the large and uncommon type of vibration known as "galloping". The effect of an ice coating on the line was investigated and could not be proved to enhance vibrations artificially introduced into the line. A mathematical expression for the period when the cable sways in the wind was developed. This period was found to be approximately equal to the period of the "fundamental" of the span. After various experiments had been carried out, the conclusion was reached which attributed the cause of galloping to pulsating winds.

Introduction

If a telephone or transmission line be viewed by an observer from a position in which his eye is on the same level as the wire, ripples can sometimes be seen travelling along the wire; or again, if one stands at the base of a steel tower, the whole tower often seems to be rattling. Both these phenomena are of common occurrence, but there is also another type of vibration which is seen only on very rare occasions and is known by engineers as "dancing" or "galloping". On these occasions the span vibrates either as a whole, or in segments, with the formation of nodes and loops. This phenomenon is of course a menace to the line, because once large vibrations start, the cable may be torn off the insulators before the motion subsides.

A very large percentage of the reports on galloping describe a coating of ice on the cable and this fact has misled engineers into formulating theories attributing the cause of these large vibrations entirely to ice. If such theories were valid, it should be impossible for galloping to occur without a coating of ice on the cable. Archbold's paper (1) in which a large number of reports on the phenomenon are assembled, clearly shows however that ice cannot be the basic cause of galloping but is merely an auxiliary agent. These large vibrations remind one so forcibly of the resonance of a stretched string to its fundamental note, that the possibility of galloping being a resonance phenomenon suggests itself at once.

The occurrence of these large vibrations is so rare that they are not of very great importance from an economic standpoint, in spite of the fact that the destruction which they may cause on a single occasion may be large; on the other hand, the continual occurrence of small vibrations is a serious matter, because eventually fatigue develops in the cable at the points of support.

The object of the investigations described in this communication was to arrive at some theoretical conclusions which would help to explain the cause of the small and large vibrations; for without the aid of theory on which to base one's reasoning, it is difficult to find suitable ways of coping with the phenomena in question.

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The Available Data

General

Before discussing the mathematics of a stretched string, it might be advisable to state briefly some information which the writer has acquired from various reports—verbal and written. Some of these reports were much more reliable than others, but generally speaking they lead one to believe the following statements:—

(a) When “galloping” occurs, ice is usually, but not always, present on the cables.

(b) “Galloping” usually occurs on lines which cross level, open country and in which the spans are of equal length and the elevations of the points of suspension are the same.

(c) A moderate wind, say about 25 m. p. h., is the most favorable wind to start galloping; gales are unfavorable.

(d) Very slackly strung lines and very tightly strung lines are both unfavorable for galloping.

The following statements are rather more doubtful:—

(e) Galloping occurs only on long lines.

(f) A wind blowing at an angle to the line is more likely to cause galloping than one blowing directly across the line.

(g) The most usual time for galloping to occur is at sunrise.

A few facts about the periodic motions which can occur in a cable when stretched between two supports are summarized below.

(1) When wind passes over a cable or other object, eddies are formed alternately on either side and cause the object to vibrate. This vibration, which may be called for convenience the “eddy” vibration, is of high frequency and is probably responsible for the rattle heard in steel towers, although this has not been proved. The diagrams in a paper by Varney (3) show that these small, rapid vibrations are superimposed on vibrations of larger amplitude and smaller frequency. However, as neither the frequency nor the amplitude of these eddy vibrations seems very suitable for building up large vibrations, they may be ruled out from the start as a possible cause of galloping. Nevertheless, as they are so frequently present, they cannot be entirely overlooked as a possible cause of the gradual fatigue found in cables at the points of support.

(2) When wind starts a cable swinging from side to side, there is set up a periodic motion very similar to that of a pendulum. This motion will be referred to as the “swing”.

(3) When a disturbance is started in a span, both ends of which are fixed, as by striking the cable with a hammer close to one end, this disturbance travels to the other end, is reflected, and thus continues travelling to and fro. The frequency depends on the distance between the supports, the mass per unit length of the wire, and the tension, and is known in the theory of sound as the frequency of the fundamental note. It will therefore be referred to as such. It is of course identical with the resonance frequency.

These three periodic motions, giving rise respectively to the eddy vibration, the swing and the fundamental note, appear to be the only characteristic vibrations associated with a span.

There may, however, be other vibrations travelling over a span and arising from various causes, for instance from the swinging of neighboring spans, or from any periodic variation of tension set up in the line by vibration from, say, a factory or power house wall to which the line is attached, or from the vibration of the poles or insulators. A most interesting case of galloping observed in a 90-ft. span crossing a railroad is cited by Archbold (1). The suggestion is that a train passing by had been travelling at a rate such that the ground around the supports vibrated in resonance with the span. The writer has produced vibration in a model span by stepping heavily on the floor—around where the wire was supported—in time with the resonance vibration of the span.

Mathematical

Considerable attention has been given to the eddy vibration by Varney (3). His attention, however, seems always to have been focused on the eddies off the cable itself, no thought being given to the eddies off the pole or, in the case of tower lines, off the chain of insulators. Obviously there must be a movement, longitudinal so far as the line is concerned, arising from the flow of air past a cylindrical body as large as a pole or a chain of insulators.

According to Varney (3), the eddy frequency for a cable is given by the equation $f = \frac{v}{D} \times 0.185$. The diameter of the cable used by Varney was one inch; therefore D , the diameter, can be put equal to $\frac{1}{12}$. The frequency, f , is thus proportional to the velocity, v , of the wind. If now we consider the frequency of vibration of a nine-inch pole instead of that of the cable, the frequencies for different winds should be $\frac{1}{9}$ of those which Varney finds for the eddy frequency of the cable. Diagrams Nos. 2, 4, 5, 7, 14 in Varney's paper show respectively the presence of frequencies $\frac{1}{7}$, $\frac{1}{10}$, $\frac{1}{7}$, $\frac{1}{8}$ and $\frac{1}{10}$ that of the cable eddy. Had the cable been strung on nine-inch poles, instead of on towers, there seems to be no reason why the motion of the poles arising from eddies should not have increased the amplitude of these vibrations. At least it can be said that the eddy frequency around the poles or insulators seems much more likely to cause large vibrations than the eddy frequency around the cable itself.

The frequency of the swing can be calculated if it is assumed, first, that the cable hangs in a catenary and that the parabolic equation applies, and second, that the wire can be treated as a rigid body. With these assumptions the radius of gyration of the span about the line joining the points of support may be calculated. The equation for the cable is given by $y = \frac{x^2}{2c}$ where c is equal to the tension divided by the weight per unit length, and y is the distance from the directrix. If h be the height of the line joining the points of suspension above the directrix, then by putting $\eta = h - y$ the equation may be transformed to axes through a point midway between the points of support. The transformed equation is in fact $\eta = h - \frac{x^2}{2c}$.

Making use of the assumption that the catenary swings as a whole, like a rigid body, the radius of gyration, k , is given by

$$k^2 = \frac{\int \eta^2 ds}{\int ds} = \frac{\int \left(h^2 - \frac{hx^2}{c} + \frac{x^4}{4c^2} \right) ds}{\int ds},$$

where ds is an element of cable length. Assuming that the curvature is almost zero, we may put $x=s$, and integrate from 0 to x_0 where $2x_0$ is the distance between the points of support. Hence,

$$k^2 = \frac{h^2 x_0 - \frac{hx_0^3}{3c} + \frac{x_0^5}{20c^2}}{x_0},$$

and since $\frac{x_0^2}{2c} = h$, because $y=0$ at points of support, $k^2 = \frac{8h^2}{15}$, so that $k = 0.73 h$.

Therefore the period of swing $= 2\pi \sqrt{\frac{k}{g}} = 2\pi \sqrt{\frac{0.73h}{g}}$.

The period of the fundamental can be taken from the theory of sound, if, as has been assumed, the curvature is negligible; this practically amounts to assuming that gravity is small compared to the tension in the line. Under these conditions the period of the fundamental may be written as $2s \sqrt{\frac{m}{T}}$ where m equals the mass per unit length and T equals the tension.

Now $2s \sqrt{\frac{m}{T}} = 2s \sqrt{\frac{W}{Tg}}$ where W is the weight per unit length. Therefore the period of the fundamental

$$= 2s \sqrt{\frac{1}{cg}}$$

where c is the constant which occurs in the equation for the catenary

$$\begin{aligned} &= 2 \sqrt{8ch \times \frac{1}{cg}} \\ &= 4 \sqrt{\frac{2h}{g}}. \end{aligned}$$

Referring back to the expression for the period of the swing, we have

$$\frac{\text{Period of swing}}{\text{Period of fundamental}} = \frac{2\pi \sqrt{.73}}{4 \sqrt{2}} = \frac{5.44}{5.66},$$

i.e. these periods are nearly the same.

The two periods are so close to each other that it was decided to arrange an experiment to ascertain if they were actually as close as theory predicted. With the aid of a stopwatch the oscillations per min. of the swing and of the fundamental were observed. They appeared to be identical. This experiment, in contrast to what usually occurs in physics, established a relation with greater accuracy than theory had indicated.

The Possible Effects of an Ice Coating on the Cable

As mentioned above, the reports indicated that a coating of ice facilitated the occurrence of galloping. Therefore, if it could be ascertained what effect ice had on a cable, it was thought that the clue to the cause of galloping would be found. Obviously, ice on the cable acts to some extent as a sail on a ship, in that it increases the total wind force on the cable without proportionally increasing the mass. The ice also forms an elastic coating on the outside of the cable and might therefore change the elastic constants. Then again under certain conditions, the ice might form on the cable as a wing and as Davison (2) has suggested, act as an airfoil. As such an ice formation is the exception rather than the rule, and as the lift forces which arise thereby are of necessity very small, the airfoil idea need not be discussed as a likely fundamental cause of galloping.

In order to investigate the possibility of a change in the elastic constants of the cable, of sufficient magnitude to cause the ice-coated cable to vibrate under conditions such that a bare cable would not vibrate, the following experiment was arranged:

By means of an electric motor, variable speed gear and eccentric, vibrations or rather variations of tension were introduced into 40-ft. span of copper wire 0.8 mm. in diameter. The variable speed gear made it possible for these vibrations to be of any frequency within certain limits, and it was therefore possible to plot the amplitude of vibration of the cable against the frequency of the exciting vibration. In this way, the resonance frequency could be found, because at that frequency the amplitude reached a sharp maximum, *i.e.*, galloping was in a sense artificially produced.

The experiment was then repeated out of doors during the winter months, in order to take advantage of the cold weather for the production of the ice-coating. A common syringe was used to make a fine spray of water resembling rain, and the ice-coating gradually accumulated on the wire where the spray struck it. The wire thus coated was then subjected to periodic variations of

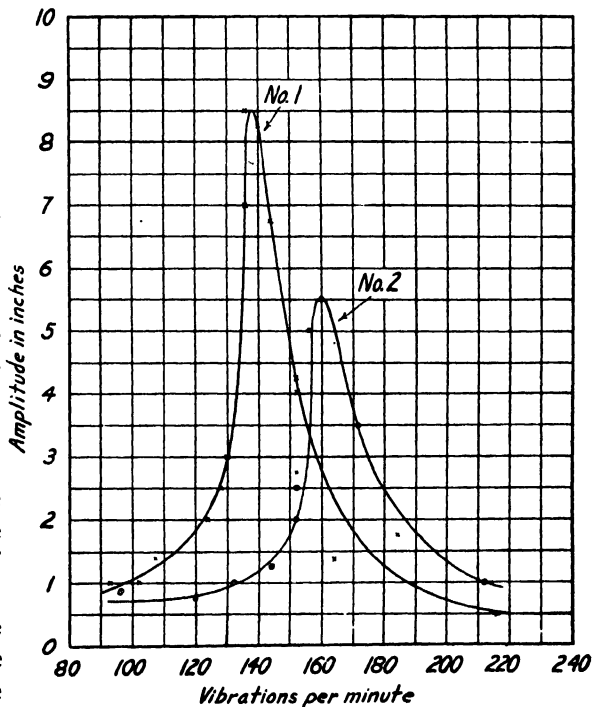


FIG. 1. Curves showing the variation in the amplitude of vibration of mid-point of span with variation of frequency of exciting vibration. No. 1—with no ice coating. No. 2—with ice coating.

tension or vibrations as before, and the amplitude plotted against the frequency. The energy of the exciting vibration was kept the same as before. The maximum amplitude when the ice was on the wire was found to be decidedly smaller than when the wire was bare, and there was no indication of tuning over a wider range of frequencies when the ice was on the wire. The ice-coated wire displayed a tendency to swing when in tune rather than to vibrate like a violin string.

In fact the experiment gave very definitely negative results, and by thus indicating that the ice-coating did not change the elastic constants in such a way as to cause galloping, it supported the other hypothesis, namely, that ice increased the effective wind force on the cable, without proportionally increasing the weight.

Fig. 1 illustrates the tuning of the exciting vibration to the resonance vibration of the span, first without any ice-coating and second with an ice-coating on the wire.

The Effect of Wind on a Span

The hypothesis that ice on the cable acts as a sail necessitated a thorough investigation of the possible effects which wind might have on the production of galloping. Wind can undoubtedly start the spans in a line swinging from side to side, and a motion thus started would theoretically cause a periodic variation of tension and so bring about a vertical motion in a neighboring span. To verify this experimentally, a three-span line was set up and the motor, referred to above, was arranged so that it gave one span a transverse, horizontal, periodic motion. The vertical motion could be observed in the other two spans, but it was much too small to account for galloping.

This result pointed to the probability that something intrinsically connected with wind was being overlooked. Therefore, merely with a view to getting an idea of what this omission might be, a wire was stretched between the legs of a table and the air stream from a common ventilating fan was directed upon it. Under these conditions the wire was found to vibrate. However, a ventilating fan gives a very turbulent air flow, so that there were undoubtedly pulses in the air stream, which were possibly the cause of the vibration. If this were so, a wire stretched in the carefully combed air stream of a wind tunnel should not vibrate; on the other hand, if the eddies referred to at the beginning of this communication, were in any way the cause of the vibration, the wire should vibrate in a wind tunnel just as readily as in front of a ventilating fan.

To settle this point, it was merely necessary to expose a stretched wire in the air stream of a wind tunnel and to ascertain whether it vibrated. In carrying out the experiment two wires were exposed to the wind. One of the wires was bare and the other was thickly coated with shellac, the latter representing ice. The air stream in the wind tunnel at the National Research Laboratories passes through a "honeycomb" grid which effectively eliminates turbulence. When the air stream flowed past the wires they bowed, but the vibration which had been observed when the wire was in the turbulent air stream from the ventilating fan did not occur. Wind velocities ranging from 20 to 60 m.p.h. were used in the experiment.

This result altered the complexion of the whole problem, for it seemed evident that it was a particular kind of wind which was responsible for the phenomenon of galloping. If this were the case, it was quite probable that the ice coating on the cable had no more to do with galloping than the fact that ice-coated cables were often associated with certain weather conditions, and these particular weather conditions were also associated with gusty winds of a particular nature. If, however, it were found that the vibration of the wire, when situated in front of a ventilating fan, increased as the shellac coating was built up, then there was evidence that in addition to the necessary wind conditions sometimes associated with sleet storms, the ice coating itself favored galloping. Accordingly the following experiment was arranged to ascertain the effect of an ice coating when the span was subjected to the action of a turbulent air stream. A three-span line was set up, the wire used being copper, 0.32 mm. diameter. The distance between the two end supports was 20 ft., and the three spans were all of the same length. A ventilating fan was placed opposite each of the three spans and the vertical motion was observed on a vertical scale placed at the middle of each span. The tension was adjusted to five ounces before the wire was finally fixed to the end supports. When the wire was bare the maximum vertical motion which could be observed was $\frac{1}{16}$ in., but after four coats of shellac had been put on, the maximum motion increased to $\frac{1}{8}$ in., and after ten coats to as much as $\frac{5}{16}$ in. The thickness of the wire was then about 1.25 mm. Clearly the shellac coating was increasing the vertical vibratory motion. It was also found that when the wire was not free to move in a longitudinal direction at the middle supports, the maximum amplitude with ten coats of shellac decreased to $\frac{3}{16}$ in. The experiment clearly indicated, first, that a coat of ice on a cable would increase any vibration caused by a gusty wind, and second, that a vibration in a span could be reinforced by vibrations from other spans, and that a long line would therefore favor the building up of a large vibration and the consequent appearance of galloping.

Summary and Conclusions

A periodic variation of tension, if it is of period identical with that of the fundamental of the span, will cause the span to gallop. In the laboratory this was produced by a motor and eccentric, but it might perhaps be produced in an actual line by vibration from a factory wall, or the vibration of the supports, caused either by air eddies from the poles or insulators, or by ground vibrations.

By virtue of the fact that the swing of a span has the same frequency as the fundamental, the swinging of spans may cause vibrations in a line in which the lengths of the spans are equal, but these vibrations are too small to account for galloping.

Ice does not increase the amplitude of vibration caused by a periodic variation of tension which may have started in the line, say through the agency of a vibrating power-house wall, but rather decreases it. Judging from the fact that in the majority of the cases of galloping, an ice coating has been reported on the cable, one might be led to conclude that ice itself causes galloping. This

seems unlikely unless ice acts as a sort of sail on the cable, but this conception implies certain kinds of gusty winds. Therefore, if suitable gusty winds must be assumed, there may be no necessity for an ice-coating at all. A thick ice coating increases the vibration caused by a gusty wind but it seems probable that the most important agent is the suitable gusty wind. As sleet is often accompanied by peculiar weather conditions, the conclusion is that the cases of galloping which writers on the subject have up to the present associated in some way with an ice coating, should really have been traced to the particular weather at the time—weather which was suitable to the simultaneous production of an ice coating on the cable and the requisite wind conditions. If such a conclusion be correct, the phenomenon of galloping is practically dependent on meteorological phenomena and further research on the subject should take into consideration particular types of wind associated with particular weather conditions. Before suggesting ways and means of preventing galloping these conclusions should be verified in the field and therefore suggestions are in a sense premature. It might, however, be possible to design some sort of air foil which could be attached to the line and which would turn in such a way, when the wind blows, that the vibratory motion would be damped. Of course, economic considerations would prevent the use of too expensive a remedy.

Acknowledgment

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A GAMMA RAY IONIZATION CHAMBER¹

BY GEORGE C. LAURENCE²

Abstract

A description is given of a γ -ray ionization chamber with its accessory electrometer box, suitable for precision measurements of radioactive preparations, and for the measurement of the absorption coefficient and specific ionizing powers in air, of γ -rays.

This ionization chamber has been designed for precision measurements of γ -radiation in the National Research Laboratories at Ottawa, where an instrument is required of sufficient flexibility to serve several purposes. It is being used for the recalibration measurements of the national radium standards of 1, 2, 5, 10 and 25 mgm. These are being compared with similar calibrations made with a gold-leaf electroscope in a search for consistent discrepancies, since the difference in method may tend to expose instrumental errors peculiar to either. It is intended for measurements of γ -ray absorption coefficients for the estimation of mesothorium impurity, for the determination of γ -ray ionization of air per unit volume, and finally, it is used for the measurement of radioactive preparations submitted to the Council for calibration and for the estimation of their mesothorium impurity. For these purposes its shape is more suitable than the usual gold-leaf electroscope. It has proved very satisfactory, yielding higher precision than is usual with the gold-leaf electroscope. This advantage is probably due to the use of a null method and long effective scale length as described below.

The ionization chamber, supported in its mounting, may be seen in Fig. 1. (Letter references in the text correspond to the wiring diagram, Fig. 2.) The radium capsule is carried in the V-shaped bucket on the left. This has a thin aluminium wall on a rigid brass frame which fits snugly in place on the top of the support, which may be clamped to the steel ferrule at the desired distance from the chamber.

Removal of the cover, visible on the back of the chamber, gives access to the collecting plate, *c*, which is of brass, 10 cm. square, and occupies a somewhat larger hole in a brass plate, *g*, 30 cm. square, which acts as a guard ring, forming a border 10 cm. wide around the collecting plate. The guard ring also forms the supporting wall of the instrument. It is bolted to the iron supporting frame, carries the box on the back, and holds the collecting plate with four quartz insulators. The face of the chamber, *d*, an aluminium plate 30 cm. square, is fastened at a suitable distance in front of the collecting plate and guard ring, on an ebonite separator, which in turn is fastened to the guard ring. This plate and separator are readily removed, so that separators of different thicknesses may be inserted, thin foils of absorption material may be put on the electrodes to reduce photo-electric emission from them, and lead absorption screens may be added as desired. The brass cup on the bottom of the box holds

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Contribution from the National Research Laboratories, Ottawa, Canada.

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a small beaker of desiccating material, and may be removed for cleaning by unscrewing. A manometer tube, hidden behind the box, Fig. 1, indicates the pressure in the chamber. In practice the cracks around the cover and elsewhere are sealed with soft wax. A block of paraffin wax is cast on the back of the collecting plate and around the connecting wire leading from it to its terminal on the left side of the box. This reduces the flow of ions to the collecting plate from the air space behind it. The connection to the electrometer is carried through the evacuated brass tube on the right.

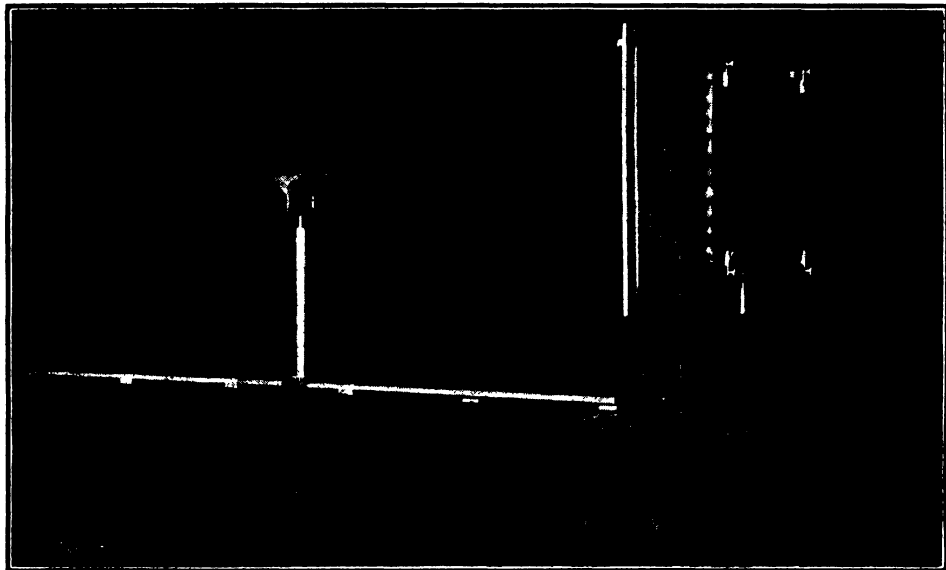


FIG. 1. *Gamma ray ionization chamber.*

The Lindemann electrometer used with the chamber is enclosed in a brass box which is evacuated by a rotary oil pump. The important details of the box are shown in Fig. 2. The reading telescope is mounted directly on the cover and a microscope mirror is supported underneath. The base is of wood and is screwed to the table. One of the four brass supporting legs is hollow and serves as an outlet for pumping out the air and the connecting leads to the electrometer are brought out through a glass pinch waxed into it. A condenser, *C*, of the coaxial cylinder type (capacity about 100 e.s.u.) is built into the tube which shields the connection to the ionization chamber. The earthing switch, *e*, is soldered into a short length of flexible corrugated metal tubing (not shown) so that it can be operated from the outside of the box. The end of the switch is shown in the drawing protruding into the chamber through its cylindrical wall. Motion in the direction indicated by the arrow brings the pin into contact with a phosphor bronze spring on the electrometer terminal (broken line) thereby earthing it.

The usual null method commonly called "tram driving" is used. The flow of ions to the collector *c* charges the inside cylinder of the condenser *C*, which

is kept close to earth potential during a measurement, by moving the rheostat R' which alters the potential of the outside cylinder of C . Readings are timed from the passage of a convenient division (near zero) in the electrometer scale with R' in the position a , to the passage of the same division with R' in the position b . Thus the collecting electrode and connection can be kept to within 0.01 volts of earth and guard-ring potential during a reading.

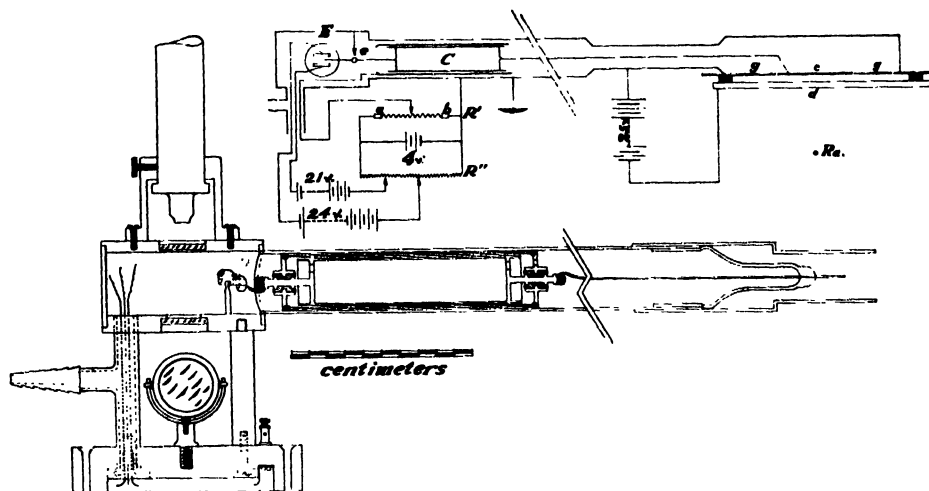


FIG. 2. Lindemann electrometer box, and diagram of connections.

The most serious sources of error in using this type of instrument may arise from the flow of ions in the electrometer, the condenser, around the connecting wire and behind the collecting plate, and from the inertia of the electrometer needle. The former is reduced by evacuation of the box containing the electrometer condenser and connection wire, and by waxing the back of the collector as described, and with care in keeping the collector system close to earth potential during measurements, it can be kept under 0.1%. The latter is kept insignificant by choosing a timing division which is a short distance on that side of the zero division to which the needle tends to move as the collector charges up, but, of course, not far enough from the zero division to introduce error from the other cause. Effects due to slow changes in conditions, such as the voltages of the electrometer batteries, are dealt with by bracketing readings.

The sensitivity in scale divisions per second for a given radiation intensity is about the same as that of a good gold-leaf electroscope; for example, a deviation of 1 division per sec. is obtained with 25 mgm. of radium at a distance of 50 cm. from the instrument. Advantage is taken of the long effective scale length made available by increasing the voltage of the condenser-charging battery, so that in practice, fewer readings of longer duration are taken than with a gold-leaf electroscope. Four volts giving an effective scale length of about 500 divisions is convenient, but larger voltages can, of course, be used. Long series of readings with the instrument have shown average departures from the mean of $1\frac{1}{2}$ per 1000.

REVIEWS AND NOTES

IMPROVEMENTS IN THE ANALYSIS OF
MAPLE PRODUCTS¹BY J. F. SNELL², LEV SKAZIN³, H. J. ATKINSON³ AND G. H. FINDLAY³

Abstract

Collaborative work on maple syrup has shown that, with refractometric control, samples can be prepared to a content of 65% solids with much greater precision than is attainable when boiling temperature alone is depended upon. As the dry basic lead acetates used in different laboratories vary greatly in solubility and alkalinity, much better concordance in Canadian lead values can be attained by use of a reagent prepared from normal lead acetate and litharge "activated" at 650-670° C. The electrical conductivity of solutions containing 25% of solids is less variable in genuine goods than any of the chemical values. The soluble ash is less variable than the insoluble.

Water Content and Preparation of Sample

Excess of water in maple syrup and sugar is objectionable not merely as representing weight without value but also because it renders the syrup more susceptible to deterioration by micro-organisms, and the sugar soft and less coherent and therefore less convenient to handle. The regulations under the Food and Drugs Act (1a) require that the water content shall not exceed 10% in the sugar, 35% in the syrup or 15% in maple butter, cream or wax. In syrup a content of water *lower* than about 32% is apt to cause sucrose crystals to separate, an effect that is commercially objectionable.

In the examination of maple products for adulteration it is usual to dilute, reboil and filter syrups and to convert solid products into filtered syrups, thus bringing into solution any soluble substances that have separated out as the result of evaporation beyond the standard syrup stage, and removing any that have remained in solution on account of under-evaporation. The aim, of course, is to bring the syrup as near as possible to the standard water content of 35% (1a, 6). As the analysis is then made with reference to this "prepared" syrup, a second measurement of water content is necessary. The determination of water (or total solids) in syrups is commonly made with an Abbé type of refractometer. It is obviously desirable that the directions for this determination should be such as will lead to consistent results in the hands of different analysts and also that the directions for preparing the sample for analysis should be so stated as to enable them to obtain products of equal water content.

In the preparation of the sample in the year 1929 the collaborators followed the directions of the 1925 edition of the A.O.A.C. Book of Methods (1), *viz.*, boiling the diluted sample to a temperature of 104° C. and filtering through

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Contribution from the Department of Chemistry, Faculty of Agriculture, McGill University, (Journal Series No. 15), with financial assistance from the National Research Council of Canada, and collaboration on the part of several laboratories under the auspices of the Association of Official Agricultural Chemists.

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³ Research Assistants.

cotton wool. Amongst 10 collaborators, none succeeded in preparing 20 syrups with a water content showing less variation than 5.8%. The average difference between the highest and lowest percentage of water in syrups prepared by each analyst was 7.7. Only two of the nine analysts who adhered to the directions prepared their syrups so that the average solids content fell within 1.3% of the standard 65%, the prepared syrups of others averaging from 57.4 to 63.7%.

As each collaborator had made his own refractometric determination of water it was not clear how much the variations in this determination in the hands of the different analysts had contributed to the differences shown in the results of preparing the syrups. In 1930, therefore, not only was an improved method of preparation of the sample proposed but analysts were also requested to report total solids in the samples as received as well as total solids after preparation. The results revealed a surprisingly wide variation in the refractometric results obtained by different analysts with identical syrups. On only half of the 30 samples examined did all collaborators report total solids in agreement within 1.0% solids. In one case the difference between the highest and lowest values reported was a little over 5%, in five between 2 and 5% and in nine between 1 and 2%.

Experiments in the Macdonald College laboratory indicated that the reading on the thermometer of the Abbé refractometer (upon which reading a correction to 20° is based) might not truly represent the temperature of the film of syrup spread between the prisms. By comparison of readings made upon a few syrups by several observers it was established that more consistent results were obtained when water was circulated through the jacket of the instrument during the observations. To obviate the possibility of dew deposition it was deemed advisable to use water of room temperature and to correct for temperature, rather than to attempt to make all readings at 20° C.

With the observance of this precaution in 1931 much more concordant collaborative results were obtained than in 1930. Amongst reports of 13 analysts on 8 syrups (104 reports) only a single one, or 1%, differed from the mean by more than 0.75% as against 7% (17 in 234) in 1930. The average deviation from the mean was 0.252% of solids, as against 0.354% in 1930.

The revised method of preparation required that when the temperature of the boiling syrup approached 104° C. the analyst should from time to time withdraw small samples, cool them and examine them in the refractometer. As a filtering medium extra rapid filter papers were substituted for the cotton wool. Two brands of paper were found which would filter 100 cc. of syrup in less than five minutes. The general average of total solids in syrups so prepared was 65.6% in 164 preparations in 1930 and 65.3% in 88 preparations in 1931, as against 61.3% in 202 preparations by the old method in 1929. The number of analysts who succeeded in preparing all syrups within a range of 1% was 1 amongst 8 in 1930 and 6 amongst 11 in 1931; within a range of 2%, 3 in 1930 and 10 in 1931; whereas in 1929 none amongst 10 had succeeded in keeping within a range of 5%. This modified method has accordingly been substituted for the old one by the Association of Official Agricultural Chemists.

Conductivity Value

The measurement of the electrical conductivity of maple syrup diluted to a definite concentration approximating that at which the conductivity attains its maximum was proposed in 1911 (12, 13, 14) as a rapid test for adulteration with refined sugar and was adopted by the Association of Official Agricultural Chemists as a Tentative Method in 1919 (7). Experience has demonstrated that the value determined by this method shows less variation in genuine syrups than any of the chemical values (15, 18, 19) and though the directions as given in the A.O.A.C. Book of Methods can probably be modified to advantage (particularly with reference to the determination of the "cell constant") the method is proving of value and will no doubt find more general use now that conductivity apparatus suitable for this purpose is being used in the sugar industry (11, 22).

First action towards the promotion of this method to the status of an Official Method was taken by the Association of Official Agricultural Chemists in 1930 (8), but final action is deferred pending revision of the directions. The solution now used is that containing 25 gm. of dry matter per 100 cc., instead of that containing 22 gm. originally recommended.

Canadian Lead Value

The measurement of the weight of the precipitate produced by basic lead acetate in solutions of maple sugar under specified conditions was proposed by the Laboratory of the Inland Revenue Department* in 1906 (9, 10) and adopted as a Tentative Method by the Association of Official Agricultural Chemists in 1919 (7). In 1928 the method was studied in detail by Fowler and Snell (2) and modified by the substitution of cold for hot water in the washing of the precipitate. Collaborative studies of this method in both forms and with the use of smaller quantities of the reagent have led to the adoption (8) of directions conforming to the Fowler modification.

The 1930 collaborative work revealed wide variations in the basic lead acetate solutions prepared in different laboratories (19) from dry lead subacetate. The alkalinities of such solutions were found to range from 6.51 to 10.14 (cc. 0.1 *N* per cc. solution), pH values from 7.1 to 7.5 and total lead per cc. from 0.1991 to 0.2397 gm. In 1931 (19a), a new method of preparing the reagent was proposed, *viz.*, activating litharge by heating to 650-670° C. for 2½ to 3 hr., and dissolving in boiling water (250 cc.) the lemon-yellow product (40 gm.) and normal lead acetate crystals (80 gm.) in the proportions used in preparing basic lead acetate solution before the dry basic acetate became a common chemical. Collaborators sent to Macdonald College not only the reagents prepared in the two ways but also portions of the solid chemicals used in their preparation.

* Mr. Thomas Macfarlane was Chief Analyst in 1906 and the method was originated at his suggestion by Mr. A. Valin, now analyst in charge at the Montreal branch of the Food and Drugs Laboratory of the Department of Health, successor to the Laboratory of the Inland Revenue Department.

Solutions prepared from these materials by a single chemist (G.H.F., who activated all the samples of litharge and also made the analyses of the collaborators' reagents as well as of those prepared by him) did not vary much from those made in the collaborating laboratories. Amongst 10 samples of dry subacetate, the greatest differences between any of the solutions prepared by collaborators and those prepared by G.H.F. were: alkalinity, 0.35 cc., 0.1 *N*; density, 0.008; and total lead, 16.1 mgm. per cc. The average differences without regard to sign were: alkalinity, 0.14 cc.; density, 0.0035; and total lead, 7.2 mgm. per cc. The range of alkalinity was slightly narrowed, from one of 5.23-11.33 to one of 5.39-11.25; the pH range (7.1-7.6) was unaffected. A solution kept over from 1930 by one of the collaborators showed somewhat greater variations from that prepared (in 1931) from the same solid by G.H.F., *viz.*, +0.62 in alkalinity, +0.014 in density and +14.6 mgm. per cc. in total lead.

Amongst nine pairs of solutions prepared by collaborators and G.H.F. from the nine samples of litharge the only differences in alkalinity greater than 0.30 were two instances in which the collaborating analysts had obtained solutions having alkalinities of 9.80 and 9.83. With these two exceptions all the collaborators' solutions fell within the range 10.11-10.51, which was practically identical with that for the solutions prepared by G.H.F., *viz.*, 10.21-10.57. The collaborators' solutions showed pH values of 7.5 to 7.6, those made by G.H.F. from 7.4 to 7.6. The total lead per cc. showed remarkably little variation in either the collaborators' or G.H.F.'s preparations (0.2316-0.2385 in the former, 0.2254-0.2328 in the latter) but was always somewhat lower in the latter.

Comparing the reagents prepared in the two ways, it was found that those made by the new method showed much less variation in alkalinity and a higher general plane of alkalinity than those made from the dry basic acetates. (In the collaborator's preparations, the average alkalinity was 10.24 for the new reagents, as compared with 8.17 for the old). With practically all the syrups they also gave higher lead numbers than were yielded by the reagents prepared from dry basic acetate. The exceptions were among the results of (a) one analyst whose dry basic acetate solution had an exceptionally high alkalinity (11.33), actually exceeding that of his activated litharge reagent, and (b) a second analyst whose litharge reagent had an alkalinity that was the lowest of its class (9.80), the alkalinity differing but little from that of his dry basic acetate reagent (9.29). The other analyst with a litharge of low alkalinity (9.83) happened to have the least alkaline of the dry subacetate reagents (5.23), so that in this case the difference between the alkalinities of the two reagents was one of the greatest found, and the differences between his lead values with the two reagents were correspondingly large.

That there is a close correlation between alkalinity of reagent and magnitude of lead value is further shown by the fact that the magnitudes of the various analysts' average differences between lead values obtained with the two reagents in the eight syrups run in the same order as the differences between the alkalinities of the two reagents. This was further corroborated by experiments which showed (a) that lower lead values were obtained by use of a

lead subacetate solution diluted with acetic acid than by use of the same solution equally diluted with water, and (b) that a series of five solutions of equal specific gravity, made with varying proportions of activated litharge and normal acetate so as to show alkalinity varying from 12.16 to 2.72, (ratios of neutral to basic lead 0.83 to 6.68) gave lead values decreasing steadily in one syrup from 4.52 to 2.12 and in another from 3.69 to 1.80.

The lead values reported for each syrup by the various collaborators were also more concordant with the reagent prepared from activated litharge than with that made from the dry salts. The average range of difference for the litharge solution was 13.2% of the average value as against 30.9% of the average with the reagents prepared from the dry salts, while the maximum range was 27.1% as against 41.5% of the average.

The ranges of the lead values found by all but two of the analysts for the eight syrups were, however, somewhat wider with the new than with the old reagent. The average range for all the analysts with the new reagent was 53.2% of the average value as against 49.7% with the old. Whether this will remain true in a larger and more varied collection of syrups remains to be determined, as does also the question whether the advantageous rapid decrease of the old lead values upon progressive dilution with refined sugar syrup (20) will characterize values determined with the new reagent. Further it is possible that a reagent with other proportions of basic and neutral lead might prove better than that in which the proportions formerly employed with ordinary litharge have been used.

Winton Lead Values

The Winton method (21), proposed practically simultaneously with the Canadian method, differs from the latter both in the use of a more dilute reagent and in the expression of results in terms of lead precipitated instead of the weight of the precipitate as a whole. Winton values are, of course, lower than the Canadian values for the same syrups, and apparently fall off proportionally to, rather than more rapidly than, the percentage of maple sugar in mixtures with refined sugar (20). The effect of modification of the method of preparation of the reagent along lines similar to those followed with the Canadian method might very well repay study.

Ash Values

In the 1929 collaboration wide variations were found in the values of total, soluble and insoluble ash, and in the alkalinities of the soluble and insoluble ash reported by collaborators. The least variable of these values appear to be the alkalinities and percentages of the soluble and of the total ash as determined upon the prepared syrups. Among 20 syrups the ranges of these values were narrower than those of the Fowler lead value and the insoluble ash values, though greater than that of the conductivity value. Further studies on these values have not been undertaken.

For further details of these studies of analytical methods, the reader is referred to the reports made to the Association of Official Agricultural Chemists and published in the Journal of that Association.

Summary

1. The outcome of work upon methods of analysis of maple syrup and sugar carried on over a period of years in collaboration with other Canadian and American chemists is summarized.

2. In the refractometric determination of solids in syrup, it is of advantage to circulate water of room temperature through the jacket of the Abbé type instrument.

3. The preparation of samples to a content of 65% of solids can be accomplished with much greater precision when observation of boiling temperature is supplemented by refractometric control.

4. For filtration the use of such filter papers as will filter 100 cc. of hot syrup in less than five minutes is recommended.

5. The electrical conductivity of solutions of maple sugar containing 25% of solids is less variable in genuine goods than any of the chemical values.

6. The dry basic lead acetates used in various laboratories vary greatly in solubility and alkalinity.

7. Reagents of much greater uniformity can be prepared from litharge "activated" at 650-670° C. and normal lead acetate. These give more concordant Canadian lead values than the solutions prepared from the dry basic acetates.

8. Of the ash values, those showing least variation in genuine goods are the amount and the alkalinity of the soluble ash.

Acknowledgment

The thanks of the authors are due to a number of chemists* in Canada and the United States for loyal and continued collaboration.

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THE MECHANISM OF POLYMERIZATION REACTIONS¹

BY WILLIAM CHALMERS²

Abstract

Attention is called to an earlier, unpublished writing by the author wherein a chain-reaction mechanism was suggested for all polymerizations leading to macro-molecular products. It is further pointed out that the only scheme of reaction which is compatible with this mechanism is that which involves only the double bond, *i.e.*, the possibility of the changes taking place by the transference of hydrogen atoms is practically excluded.

The appearance of papers by Milas (3), Starkweather and G. B. Taylor (4) and, more recently, by Hugh S. Taylor and Vernon (5) and by Conant and Peterson (2) seems certain to establish a chain mechanism in several polymerization reactions. That all polymerizations of olefinic compounds leading to the formation of macro-molecular bodies take place by a chain reaction was proposed by the writer in a report to the National Research Council in February 1930 (1, pp. 32-33). This study was based upon semi-quantitative work done by various chemists and will be elaborated in a paper which will appear in the near future. The following brief account differs only slightly in mode of presentation from that given in a section on the "Kinetics of Polymerization" in the report mentioned.

The key to the nature of the reactions is given by the observation that only traces, if any, of lower polymers are formed. Particularly is this apparent in the photo-polymerization of the vinyl halides and vinyl cyanide where the deposition of polymer commences almost immediately on exposure to the activating radiation, although the residual monomer does not alter appreciably in properties. A reaction of the following type is clearly indicated:

$\text{CH}_2:\text{CR}_1\text{R}_2^* \longrightarrow \text{CH}_2:\text{CR}_1\text{R}_2 \text{ (activated)} \text{—at a rate comparable with those of the ordinary reactions of organic compounds—}$

$\text{CH}_2:\text{CR}_1\text{R}_2 \text{ (activated)} + (n-1) \text{CH}_2:\text{CR}_1\text{R}_2 \longrightarrow (-\text{CH}_2-\text{CR}_1\text{R}_2-)_n \text{ (polymer)—practically instantaneous—}$

The application of this chain mechanism is not confined to photo-polymerization but applies to similar changes under the influence of heat and catalysts.

In the simplest case of such a reaction, a certain fixed proportion of the molecules becomes activated at any moment. That is to say, the rate of formation is proportional to the concentration of monomer. The velocity of *activation* is

* $\text{CH}_2:\text{CR}_1\text{R}_2$ is adopted as a general formula for olefinic compounds showing a tendency to transformation to high-molecular polymeric forms, R_1 representing an unsaturated (negative) group or a halogen atom and R_2 a hydrogen atom, alkyl group, or another "negative" substituent.

¹ Manuscript received May 6, 1932.

² Holder of scholarships under the National Research Council of Canada, 1927-1931, at McGill University, Montreal, Canada, and the University of Freiburg, Germany.

given by the familiar unimolecular equation,

$$\left(\frac{dx}{dt}\right)_1 = k(a-x),$$

(where a is the initial concentration of monomer and x molecules of monomer are converted into the polymer at the end of time t). It seems to hold true, at least over a large range, that the (average) order of polymerization does not vary materially during the course of the reaction. If this factor be represented by n , the velocity of *aggregation* will be given by the expression:

$$\begin{aligned}\left(\frac{dx}{dt}\right)_1 &= n \cdot \left(\frac{dx}{dt}\right)_1 \\ &= kn(a-x) \\ &= K(a-x).\end{aligned}$$

Thus the reaction will be pseudo-unimolecular.

While in many instances complications are introduced by auto-catalysis, as well as by side reactions, at least several known cases approximate to such simple behavior.

With a knowledge of the actual nature of polymerization reactions we are enabled to proceed to an important conclusion which may be outlined briefly as follows. The formation of the macro-molecule takes place at a rate comparable with the ionic reactions of inorganic chemistry. Any theory, then, which pictures the polymerization of styrene and related compounds as taking place in distinct stages by the transference of hydrogen atoms is rendered highly unlikely. The only type of reaction which can be brought into accord with the observed kinetics is that in which the double bond (more accurately, the carbon atoms bound by the double linkage) alone takes part. This would require only the rearrangement of electrons and can be readily pictured as taking place with a speed far exceeding any of the ordinary reactions of organic chemistry where atomic transfer is involved.

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ON THE TESTING OF FIBRE-BOARD INSULATION¹

By C. D. NIVEN²

Abstract

A description is given in detail of the hot-plate apparatus which has been installed at the National Research Laboratories at Ottawa, primarily with a view to ascertaining the cause of the inconsistency in results obtained by different investigators on insulating wall board. Fibre boards composed either of bagasse or wood fibre were tested, and a migration of moisture from the hot to the cold side was found to occur. The conductivity of a board with a fairly high moisture content was found to decrease enormously during a long period of testing. The work indicates that the conductivity-density relation is essentially linear, but should be represented as a band rather than a line to allow for the effect of the structure of the board on the conductivity.

Introduction

The determination of the heat conductivity of fibre board has become a problem of considerable interest in connection with the heat insulation of houses. The apparatus used for this determination is known as the hot-plate apparatus and consists essentially of an electrically heated plate and two cold plates cooled by a circulation of tap water or brine. This type of testing apparatus has been accepted in the United States as the standard apparatus for measuring the heat conductivity of building boards, and a design described by Van Dusen (6) is used by the Bureau of Standards at Washington.

It is to some extent unfortunate that, when the hot plate was accepted as the standard apparatus, the design of the apparatus and the conditions of test were not specified in detail. The consequence of this lack of specification has been confusion in the results: almost every conceivable suggestion has been made to explain the discrepancies, even to the extent of questioning the truth of Fourier's law. It was mainly with a view to discovering where the difficulty lay in making these tests that the work described in this communication was undertaken.

The following suggested themselves as reasonable explanations for the confusion in the results: first, the apparatus used by some of the experimenters was of unsuitable design and therefore gave erroneous results; second, there actually were variations in the samples of materials used for the tests, although

¹ Manuscript received May 10, 1932.

² Contribution from the National Research Laboratories, Ottawa, Canada.

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these samples were ostensibly the same in so far as trade name was concerned; third, the experimental procedure varied in the different tests; fourth, the moisture content varied in the different samples tested; and fifth, in the period of the tests, thermal equilibrium had not been reached. The recent paper by R. Ruedy (5) shows that the last possibility can be ruled out as it should theoretically take less than two hours to come within 0.1% of the final value.

Apparatus

General

Before deciding on the design of the hot plate to be installed at the National Research Laboratories at Ottawa, various types of apparatus described in the literature were considered. The design of Van Dusen appeared to be both simple and accurate, and was therefore taken as the model. A complete description of this apparatus has been given by Van Dusen (6) and need not be repeated here. The following slight changes were made: the overall dimensions were increased from 12 by 12 in. to 18 by 18 in.; the width of the guard ring on the outside of the hot plate, *i.e.*, the copper guard plate, was increased from 2 to 3 in.; and the width of the bakelite, on which the guard-ring element was wound, was increased from $\frac{1}{2}$ to 1 in.

These modifications necessitated changes in the spacing of the heating element: thus, along one edge the spacing was 1 in. but along the edge at right angles the spacing was 1.029 in. This figure was determined by dividing 18 in. into $17\frac{1}{2}$ parts. The ribbon used in winding had a resistance of about 0.15 ohms per ft., giving a total resistance under the central square foot of the

hot plate, *i.e.*, under the effective area, of slightly more than five ohms. The thickness of the copper plates used on the outside of the hot plate, *A*, was $\frac{1}{8}$ in., and the thickness of the bakelite on which the constantan ribbon was wound was $\frac{3}{16}$ in. The total thickness of the hot plate was thus about $\frac{7}{16}$ in. (See Fig. 1).

The cold plate consisted essentially of an aluminium casting about 1 in. thick. In this was cast a channel 2 in. wide and $\frac{1}{2}$ in. deep, following a labyrinthine course from the centre to the edge. A plate of copper $\frac{1}{8}$ in. thick covered the channel and was held tightly against the aluminium casting by means of countersunk brass screws in the

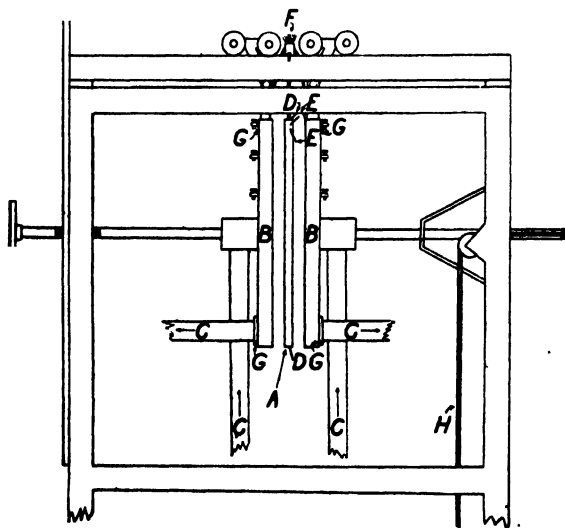


FIG. 1. Diagram of hot-plate apparatus: *A*, hot plate; *B*, cold plates; *D*, current leads to main heating coil; *E*, current leads to guard-ring heating coil; *F*, nine thermocouple-lead wires from hot and cold plates; *G*, studs used in thickness measurements; *H*, cable suspending weight; *C*, brine pipes.

web between the turns of the channel. The copper face of the completed cold plate was placed next to the sample. A little lacquer was found to be effective in preventing leakage of the brine past the screwheads. To avoid the trapping of gas at the top of each turn of the channel, pet cocks were screwed into the casting to provide escapes.

In order to press the plates and samples together during the test with the same pressure each time, two push bars were arranged at opposite sides of the apparatus to press against the middle of each of the cold plates. One push bar was screwed through the frame of the apparatus, while the other was pulled inwards by two cables passing over pulleys and carrying a heavy weight. This weight exerted a pressure on the plate of 35 lb. per sq. ft.

To heat the main element of the hot plate, current was supplied from a group of five six-volt batteries. These batteries had to be carefully charged, and it was found necessary to have a spare set; the one set could then be charging while the other was discharging. The guard-ring element was heated by direct current from a generator, while the cold plates were cooled by brine pumped through them from a tank connected to a refrigeration machine.

Calibration

The measurements of heat conductivity on a hot-plate apparatus involve essentially three quantities, the heat energy supplied per hr. per sq. ft. of surface, the temperature difference between the hot and cold surfaces which this energy supply can maintain, and the thickness of the insulator separating the two surfaces. The first of these three—namely, the energy supplied per hour—involves not only the measurement of the current but also of the resistance per square foot under the effective heat transmitting surface. It is in order to eliminate the complication of a resistance varying with temperature that the constantan ribbon is used in this type of hot-plate apparatus.

In order to measure accurately the resistance under the effective surface, the following procedure was adopted. The hot plate was put together without inserting the micanite insulation between the copper plates and the heating element. The centres of the small portions of ribbon, exposed to view in the saw cut separating the central and guard ring portions of the copper plate, were marked by drawing a pencil round the saw cut. The plate was then taken apart and the resistance of the pieces of ribbon between the pencil marks was measured. The potentiometer method was employed, so that it might be possible to use point contacts, pressing on the pencil marks, for the potentiometer-lead contacts.

The values of the resistance measurements thus obtained were then added together in order to ascertain the total resistance under the effective heating surface. This figure was checked against the fraction of the resistance of the whole heating element, obtained by measuring its resistance and multiplying by the ratio of the area under the central portion of the copper plate to the total area of the bakelite plate, taking care to allow for the edges of the bakelite plate, and the width of the saw cut. An additional check was made by measuring the lengths between the pencil marks and multiplying by the specific resistance of the ribbon.

When the total resistance had been accurately ascertained, the resistance per sq. ft. was calculated by dividing by the area enclosed by the pencil mark in the saw cut. When this figure is multiplied by the constant 3.4145 and by the square of the current measured in amperes, it represents the number of B.T.U.'s per hr. supplied per sq. ft. of surface area. The accuracy of measurement of the thermal energy supply is also dependent on the accuracy of current measurement. The measurement of the latter is by no means simple on account of the difficulty in keeping the current constant. It is necessary to watch the current reading on an ammeter during the test, making frequent adjustments of the rheostat in series with the heating element in order to maintain the current at a constant value. With the equipment used by the writer errors of about 0.3% were possible so that, due to errors in current measurement alone, an error of 0.6% might be introduced in the final expression for conductivity owing to the fact that current appears as the second power in this expression.

The second important quantity to measure is the temperature difference. This was measured by means of a copper-constantan thermocouple, *F*, one of the two junctions being fixed to the hot plate and the other to one of the cold plates. The thermocouple e.m.f. was measured on a Leeds and Northrup type K potentiometer. Readings could be taken with an accuracy of about 0.1°F. on a temperature difference of 60°F. The possible error, therefore, which might arise from faulty measurement of temperature difference appears to be small. The real accuracy, nevertheless, depends not only on the accuracy of the original calibration of the thermocouples, but also on the freedom of the apparatus from parasitic e.m.f.'s. The thermocouples used were accurately calibrated over the range in which the tests were to be made and the points were plotted on the same graph as the calibration values of L. A. Adams (1). The writer's calibration was within 0.25% of that of Adams.

The freedom from parasitic e.m.f.'s depends on the efficiency of the micanite insulation between the heating element and the copper plate to which one junction of the thermocouple is attached. If the electrical leak is to be negligible when the 110-volt line is connected to the heating circuit, this insulation must be efficient. In the apparatus described above, the resistance between the plate and the heating element measured more than 10 megohms. After the final readings on the thermocouples have been taken in an actual test, it has been found to be a good plan to cut off all the electrical circuits and ascertain if there is any change in the thermocouple reading. If there is none the micanite insulation is satisfactory.

It is very important to maintain the temperature of the cold plates at a constant known value, in order that the thermocouple reading may be converted to temperature difference, since the e.m.f.-temperature curve is not strictly linear.

The third essential measurement in making a test is the thickness of the sample. To measure this thickness the method employed by the Bureau of Standards was used. Into each of the cold plates, at the corners, four studs were screwed permanently. After cleaning any rust off the heads of these

studs and dusting the faces of the plates themselves, the push bars were allowed to draw the apparatus together. The distances between the four pairs of studs were then measured on vernier calipers. The sheets were then put in between the plates and the distances again measured. Half the mean of the four differences between these two sets of measurements gave the effective thickness of a sheet. In measuring soft materials the push bar should be clamped so that the constant pressure during test will not change the thickness of the sample. It is rather difficult to estimate the error in the measurement of thickness as it is possible to repeat a measurement on one-inch material to 1/1000, yet in many cases, for no apparent reason, measurements have not repeated to even 5/1000. It does not seem justifiable to conclude that the calipered measurement is at fault when the figures do not repeat. Obviously the position and orientation of the sheets with regard to the apparatus might have changed and so caused a change in the measured thickness. This would be accompanied by a change in the heat insulation due to the small additional air space between the sheets and the plates, which, as Van Dusen points out, may be considered for all practical purposes as having the same conductivity as a wall board. This would result in a change in the total heat resistance without necessarily altering the specific heat resistance of the sample to any appreciable extent. In short, when a measurement of thickness on the same board containing the same amount of moisture will not repeat, there may be a corresponding change in the energy-supply measurement, which to a large extent cancels what might otherwise be considered as an error.

Operation

The accuracy of the three measurements discussed above, namely, energy supply, temperature difference, and thickness, determines the calibration of the apparatus, but however carefully these measurements are made, the results which the apparatus yields will not be accurate unless the experimenter makes certain that temperature equilibrium has been reached when the final readings are taken, and that, for some time before the equilibrium readings are taken, there has been no lateral loss of heat from the effective portion of the hot plate. -

It is in order to prevent any lateral flow of heat that the apparatus is equipped with a guard ring. Van Dusen (6) explains the method of determining when the temperature of the guard ring is the same as that of the main portion of the plate. The method consists of adjustment of the guard-ring current until there is a zero reading on a number of thermocouples in parallel, the junctions of which are on the guard ring and on the main portion of the hot plate. It is as essential to watch this thermocouple reading closely as it is to maintain a constant current through the main heating element.

Mainly on account of the difficulty connected with maintaining electrical currents absolutely constant over long periods the following method was used to obtain the final readings. The current was set and maintained at a particular value after the guard-ring current had been adjusted to maintain a zero value on the guard-ring thermocouples. If the temperature difference between the

hot and cold plates increased, the main current was cut down and if the temperature difference decreased the main current was increased. In this way the current could be adjusted around a value above which the temperature difference increased and below which the temperature difference decreased. By narrowing down the limits the equilibrium current for a particular temperature difference was obtained. Obviously, when the limits had been narrowed down the importance of maintaining a zero reading on the guard ring thermocouple was increased. This method of obtaining the equilibrium readings cannot of course be applied until the equilibrium temperature gradient has been nearly established, and therefore overheating the hot plate at the start speeds up the test.

The Effect of Moisture in Samples under Test

In order to compare the results of observations on boards of different densities conductivity was plotted against density. The results for the two kinds of wood-fibre board and for bagasse-fibre board all lay on a more or less smooth curve, but there were features about these results which were not altogether satisfactory. On a two-inch board it was found that after 48 hr. of testing the value for the conductivity was still changing and the question at once arose as to whether six hours had been a long enough period of test for the one-inch sheets. Experiments lasting about 24 hr. were carried out to check the results of the six-hour tests, and it was found that while the results on a cork board sample, measured after 6 hr., could be almost repeated after 24 hr., the results on ordinary fibre boards changed considerably according to the duration of the test.

During the summer it had been noticed that the fibre boards were gaining in weight while the weight of the cork board remained approximately constant. This suggested that the moisture content was in some way connected with the change in conductivity after long periods of testing, pointing to the necessity of making a systematic investigation of the variation in conductivity of fibre boards containing different amounts of moisture. A humidifying chamber was constructed so that boards would be in a moist atmosphere without being in contact with water and the following series of experiments was then conducted on the fibre boards.

The board was first taken from a collection which had been lying in the atmosphere of the room, and was subjected to the heat-conductivity test; equilibrium readings were taken after about 6 hr. and again after about 24 hr. The board was then taken out of the testing apparatus and dried, the conductivity test repeated and the board was then put into the humidifying chamber for several days. After there had been a considerable gain in weight the conductivity test was repeated. Each time, the boards were weighed before and after testing. During a test the weights of boards with high moisture contents decreased and the weights of very dry boards increased. The conductivities after 6 and 24 hr. were plotted against the weight before testing and diagrams, of which Fig. 2 is typical, were obtained. Clearly, the duration of test was all-important, and from the fact that a board which was initially

dry gained in weight while a board which was initially high in moisture lost in weight, the suggestion was that the moisture in the board migrated in such a way as finally to set up a moisture gradient. One test was particularly interesting; a wood-fibre board, initially containing as much as 13.1% of moisture, was tested, and the equilibrium readings were taken after 6, 24, 48 and 72 hr. in the hot-plate apparatus. The conductivities were 0.380, 0.367, 0.354 and 0.349 respectively. When the board was taken out of the apparatus there was a thin sheet of ice on the surface next the cold plate; yet in spite of this fact, the weight of the board had decreased during the test.

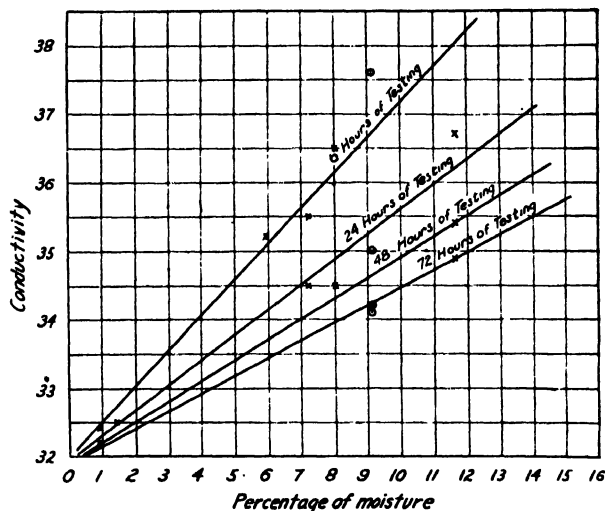


FIG. 2. Relation between conductivity and percentage of moisture in boards under test: X, temperature 43° to 53°F. ; \odot temperature 67° to 71°F. The ordinates of the points of intersection of radial lines with vertical lines indicate conductivities after various times.

There was no further doubt as to what was happening. Moisture was migrating from the warm side of the board to the cold side and the effect was virtually the same as a gradual drying of the warm side. The sheets which were dried in the oven were dried at only 150°F. and may have retained a little moisture, thus accounting for the slight variation with length of test, even on the dried sheets.

When these diagrams were obtained, the necessity of reviewing the whole subject became more apparent than ever. The presence of moisture was not only affecting the conductivity of a sample by changing, so to speak, its physical properties generally, but was also bringing in a time factor due to the gradual migration of moisture from the hot to the cold side. The question then arose as to whether the 6-hr. readings or the 24-hr. readings gave the true value of thermal conductivity. The 24-hr. readings were probably the more reliable, yet the 6-hr. readings gave a more real measure of the conductivity of a board with a particular moisture content. This leads to the conclusion that the hot plate is not the ideal apparatus; in actual use a wall board must be continually changing its moisture content, depending on the humidity of the room. As, however, the hot plate is the only apparatus so far developed which has proved even tolerably satisfactory, the only thing possible at present is to tabulate the results giving details of the various factors involved in each particular case.

The Effect of Mean Temperature

The conductivity of a fibre board increases as the mean temperature during test is raised. To express the relation between mean temperature and conductivity, Van Dusen and Finck (7) used the equation

$$K_t = K_{90}[1 + \alpha(t - 90)],$$

where K_t is the conductivity at temperature $t^\circ\text{F.}$ and K_{90} at 90°F. , t is the mean temperature of the sample under test, measured in $^\circ\text{F.}$, and α is a constant referring to the particular material in question. In order to obtain data which will determine α it is necessary to have two mean temperatures sufficiently far apart that the experimental error may not mask the small change in conductivity with temperature. It was necessary, therefore, to use a very much larger temperature difference in obtaining the reading at higher temperatures than that which had been used in the rest of the work, because there was no adequate equipment on hand to raise the temperature of the cold plate sufficiently.

TABLE I
VALUES OF α FOR THE EQUATION OF FINCK
AND VAN DUSEN (7) AS DETERMINED BY
THE AUTHOR AND BY FINCK AND
VAN DUSEN

Material	Value of α as determined by:	
	Finck and Van Dusen	Author
Bagasse	0.0013	0.00096
Bagasse	0.0012
Bagasse	0.0012
Wood fibre No. 1	0.0013
Wood fibre, low density	0.0012
Wood fibre No. 2	0.0010
Balsam wool	0.0009
Fibrofelt	0.0011
Insulite	0.0020

The values of α found by the writer and shown in Table I are in satisfactory agreement with the values given by Van Dusen and Finck (7) and clearly indicate that a variation of 10°F. in mean temperature does not make any significant difference in the results, at least, not unless the experimental error can be reduced below 2%. There was, however, a question as to whether a test carried out with a cold-plate temperature below 32°F. would give results differing from a test with a cold-plate temperature well above 32°F. This was especially important with regard to the migration of moisture, as it was suggested that perhaps a low cold-plate temperature favored this phenomenon.

A test was made on a specially humidified board, and the same gradual decrease in conductivity was observed during a 72-hr. test that had been observed during a similar test with a cold-plate temperature below 32°F. The results of this test are shown in Fig. 2. It may be concluded therefore that the migration of moisture is primarily dependent not on the temperature of the cold plate, but on the temperature difference maintained between the hot side and the cold side.

Tabulation of Results

The following have been tabulated in Tables II and III: material, thickness, density, moisture content, duration of test, mean temperature, temperature difference and conductivity. The temperature and humidity of the room

during test are not of great importance, except in so far as they may under exceptional conditions upset a test; for instance, certain very hot days inter-

TABLE II

DATA FROM HEAT-CONDUCTIVITY EXPERIMENTS WITH VARIOUS INSULATING BOARDS, COLD-PLATE TEMPERATURE BELOW 32°F.

Thick- ness, in.	Moisture %	Den- sity, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ductiv- ity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ductiv- ity
Wood fibre, No. 1, ½ in.*										
0.540	6.7	18.2	6	46	55	0.395
0.532	6.7	18.2	6	44	54	0.390
0.532	6.7	18.4	5	46	57	0.381	24	47	56	0.377
0.518	0.0	17.6	6	45	57	0.357	24	46	57	0.357
Wood fibre, No. 1, 1 in.*										
0.815	6.8	18.5	5	49	56	0.405
0.816	7.4	18.8	5	46	57	0.406	23	47	56	0.400
0.797	0.8	18.0	6	45	56	0.377	25	45	55	0.370
Wood fibre, No. 1, low density, 1 in.*										
0.993	7.3	10.6	6	45	57	0.344	29	45	59	0.331
0.979	0.8	10.0	6	44	56	0.325	24	48	57	0.320
0.989	6.7	10.6	5	42	57	0.347	24	42	57	0.336
0.979	0.4	9.98	6	45	56	0.323	27	44	56	0.318
Wood fibre, No. 2, ½ in.*										
0.431	3.8	16.6	6	45	58	0.369
0.525	3.8	16.7	6	45	54	0.369
0.526	?	15.1	6	43	55	0.362
0.563	4.8	15.1	6	44	55	0.362
0.530	5.6	15.4	6	44	54	0.362
0.475	3.9	17.1	6	44	53	0.362
0.538	7.5	13.7	5	45	53	0.347	27	43	51	0.342
0.530	5.4	16.2	5	48	55	0.366	25	46	53	0.358
0.529	7.2	15.8	5	45	54	0.360	26	43	50	0.362
0.516	0.0	15.0	5	46	55	0.339	24	44	52	0.339
0.535	10.2	16.1	6	46	55	0.378	26	44	52	0.367
0.521	5.6	17.1	6	43	56	0.364	25	48	55	0.359
0.530	7.9	17.1	30	50	53	0.375
Wood fibre, No. 2, 1 in.*										
1.115	5.9	16.0	6	43	54	0.352
1.116	8.0	13.4	6	46	51	0.365	29	48	53	0.345
1.092	1.4	12.7	26	49	57	0.325
1.089	0.9	12.7	5	45	54	0.324	29	44	54	0.322
1.132	11.6	13.7	5	48	55	0.380	30	47	56	0.367
1.116	7.2	13.2	34	53	55	0.345

*This is nominal thickness, the exact thickness is shown in the column headed, "Thickness".

TABLE II—*continued*

Thick- ness, in.	Moisture %	Den- sity, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ductivity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ductivity
<i>Wood fibre, No. 2, loose</i>										
1.099	5.1	5.0	5	44	56	0.311	24	43	54	0.304
1.075	5.1	3.1	7	45	56	0.290	27	44	57	0.284
1.095	5.1	7.3	23	45	56	0.298
1.092	5.1	7.3	5	44	56	0.307	25	47	55	0.298
<i>Bagasse, ½ in.*</i>										
0.472	5.8	15.5	6	43	54	0.361
0.471	5.8	15.3	6	42	54	0.362
0.470	6.4	15.5	5	45	56	0.353	28	47	56	0.348
0.456	0.0	14.9	6	44	56	0.334	25	46	56	0.333
<i>Bagasse, 1 in.*</i>										
0.897	6.6	18.2	6	48	55	0.403
0.879	1.4	17.8	6	47	55	0.373	29	48	56	0.363
0.920	9.6	18.3	6	45	56	0.396	29	48	55	0.381
0.895	7.4	18.4	29	53	59	0.375
0.881	1.3	17.8	29	49	53	0.361

*This is nominal thickness, the exact thickness is shown in the column headed, "Thickness".

fered with the cooling; or, again, accumulation of moisture on the edges of the plates and samples on a humid day might possibly interfere with the thermocouples. This was largely obviated by the use of pads placed around the edges of the plates so that the moist air was kept away from the material under test. To a large extent the moisture content of a sample was dependent upon the particular date of testing and the humidity at that time. The temperatures of the hot and cold plates can be calculated from the temperature difference and mean temperature data. The items which have been tabulated are too numerous to permit of expressing the results in graphic form without making certain compromises. Conductivity has been taken as the dependent variable, and density, which is the most important of the other items, has been taken as the independent variable. Moisture content and mean temperature have been taken as parameters, and thickness and particular type of material have been ignored. The influence of these factors will be discussed briefly below; only the results on tests conducted during the day and continued overnight have been plotted.

The three lines on Fig. 3 relate to tests at different temperatures on dried samples, while Figs. 4 and 5 relate to samples containing moisture. Obviously the points do not lie strictly on the straight lines even when the data have been

TABLE III

DATA FROM HEAT-CONDUCTIVITY EXPERIMENTS WITH VARIOUS INSULATING BOARDS, COLD-PLATE TEMPERATURE ABOVE 32°F.

Thick- ness, in.	Moisture %	Den- sity, lb. per cu. ft.	Single day tests				Overnight tests			
			Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ducti- vity	Duration of test, hr.	Mean temp. °F.	Temp. diff. °F.	Thermal con- ducti- vity
Wood fibre, No. 1, low density, 1 in.*										
0.975	0.5	10.0	5	62	56	0.327	25	63	55	0.330
0.980	3.8	10.4	6	67	56	0.342	30	67	56	0.327
0.974	0.6	10.0	6	67	55	0.330	25	67	56	0.325
0.990	7.5	10.6	5	67	56	0.361	24	67	55	0.341
0.970	0.0	10.0	5	72	56	0.321	25	70	53	0.327
0.970	0.1	10.0	6	98	108	0.342	30	98	111	0.346
Wood fibre, No. 1, 1 in.*										
0.797	0.8	18.0	6	68	56	0.382	24	65	55	0.377
0.793	1.1	18.1	5	70	56	0.377	25	69	54	0.371
0.793	0.0	17.9	5	97	101	0.394	24	103	89	0.399
Bagasse, 1 in.*										
0.898	5.2	17.9	6	67	56	0.373	25	66	54	0.371
0.879	0.3	17.5	6	66	54	0.360	25	66	54	0.361
0.875	0.2	17.5	6	71	54	0.362	25	71	54	0.363
0.875	0.0	17.5	5	92	96	0.374	25	93	99	0.377
Bagasse, ½ in.*										
0.467	4.7	15.3	5	66	51	0.347	24	64	50	0.348
Wood fibre, No. 2, 1 in.*										
1.117	9.1	13.6	5	68	55	0.376	24	71	59	0.350
1.083	0.3	12.7	6	98	104	0.353	30	110	107	0.344
1.081	- 0.0	12.7	6	69	54	0.329	25	68	54	0.328

*This is nominal thickness, the exact thicknesses are shown in the column headed "Thickness".

carefully classified in this way. It is also apparent that the materials of the highest and lowest densities have conductivities considerably greater than one would expect if there existed a linear relation between conductivity and density. At first sight one might be tempted to draw a curve through these points and conclude that at high density the conductivity rose rapidly and that at low density it tended to approach a constant value. In a paper by Finck (4) curves are given showing the relation of density to thermal conductivity for wood pulp and bagasse. Both of these curves indicate that the relation is not linear, but on closer examination it is seen that the curve for wood pulp is constructed from points whose distances from points determining

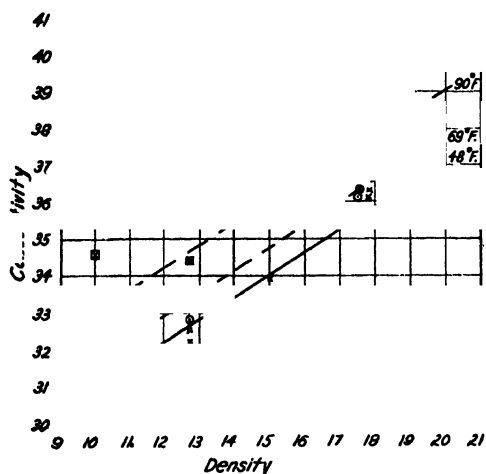


FIG. 3. Relation between conductivity and density in lb. per cu. ft. of boards under test: X, temperature 44° to 49° F.; O, temperature 65° to 72° F.; \square , temperature 93° to 110° F. Samples dried in oven at 150° F.

same diagram as some data on the trimmings of one of the wood-fibre boards. The trimmings were packed at different densities. This diagram seems to show that the linear relation holds good between the densities of 3 and 18 lb. per cu. ft. The departures from linearity amount to as much as 5 or 6%. It is interesting to note that those materials which have conductivities considerably higher than that which the linear relation implies are one-inch boards. The deviation from the linear is greater than any detectable difference between a bagasse and a wood-fibre board of the same density, and there is therefore considerable justification for plotting results for different fibre boards on the same graph.

The fibres of bagasse raw material are longer than wood fibres and would therefore be more likely to lie in the plane of the board than perpendicular to it. In thick boards the tendency for the fibres to lie in the plane of the board might decrease, especially if the fibres were short. This probably

a linear relation are less than the experimental error. In the case of the bagasse curve, Finck's work indicates a very definite trend which would imply that bagasse of density 18 lb. per cu. ft. would have a conductivity of 0.45. This is hard to reconcile with work done on bagasse board.

Some data on wood-fibre board trimmings tested by the writer, when combined with the data on actual boards, point to the conclusion that the density-conductivity relation is essentially linear, and that the departures from the straight-line relation are due to packing or structure. Fig. 6 shows the data for fibre boards of various materials containing between 5 and 8% of moisture, plotted on the

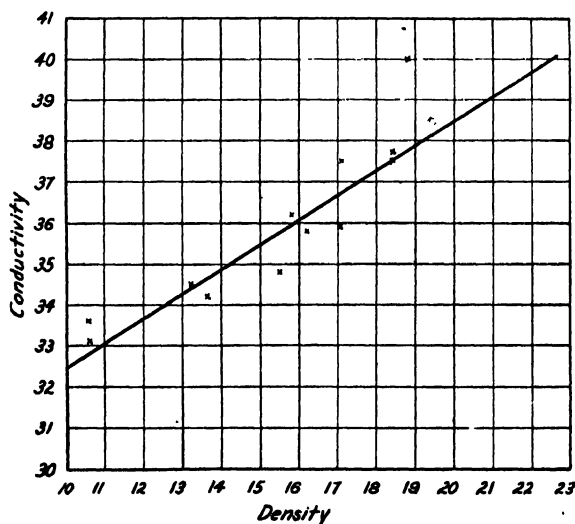


FIG. 4. Relation between conductivity and density of boards under test: temperature, 42° to 53° F.; moisture, 6.7%.

accounts for the fact that the half-inch wood-fibre board of density 17.6 has a lower conductivity than the one-inch bagasse board of density 17.8, while the latter again has a lower value than the one-inch wood-fibre board of density 18.0. The thickness, structure, and material of the board are so interlocked in their effect on conductivity that it would be unwise to differentiate between the insulating qualities of bagasse and wood fibre without carrying out a large number of tests on numerous boards taken at random from stock. Care would have to be taken in such a series of tests to see that the boards were of the same density and of the same nominal thickness; *i.e.*, a half-

inch board should not be compared with a one-inch board planed down to half an inch. If the tests were not performed on bone-dry material it would be essential to see that the moisture contents of the materials under comparison were the same.

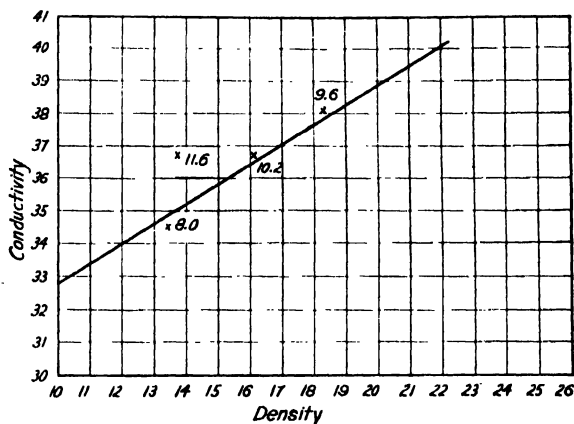


FIG. 5. Relation between conductivity and density of boards under test: \times , temperature 44° to 48° F.; moisture about 9.4%. The number written in at the point indicates actual moisture content of sample.

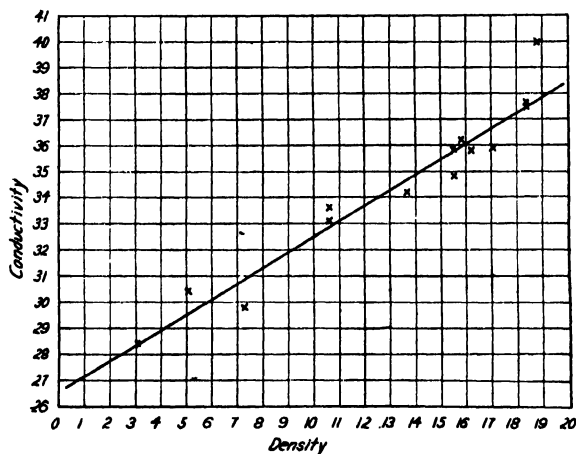


FIG. 6. Relation between conductivity and density: temperature, 42° to 53° F.; moisture 5 to 8%.

conductivity of a body is determined, not by the size of its component particles, but by their shape, and by the way in which they are packed. Reference might also be made to Finck's paper (4) on the mechanism of heat flow in fibrous materials, in which it is shown that the conductivity is greater when the fibres are packed along the direction of heat flow than when they are packed at right angles. Finck's work explains why a thick board may have a higher conductivity than a thin board composed of the same material and packed at the same density, while Awbery's work explains why the compara-

tively large bagasse fibres, when compressed to form a board, give a resultant thermal conductivity differing very little from that of a board composed of very small wood fibres, provided of course that the density of the finished board is the same in either case.

Comparison with Other Results

In order to compare the results described above with those of other experimenters, Table IV has been constructed. Column 5 contains the values which the writer's work leads him to believe are representative of fibre boards of particular densities. They have been arrived at by examining the graphs on Figs. 3, 4 and 5, and by using the equation $K_t = K_{90} [1 + \alpha (t - 90)]$. To convert a value for a sample containing 5 to 8% of moisture to the value for the dry sample, a reduction of 0.014 has been made on the heat conductivity value. This figure is obtained by subtracting the value of the conductivity for a particular density, as given in Fig. 3 by the line marked 48°F., from that given in Fig. 4 for the same density.

By applying these corrections for temperature and moisture, the results are in a form in which they can be compared with those of Finck. The agreement

TABLE IV
A COMPARISON OF HEAT-CONDUCTIVITY VALUES OBTAINED BY THE AUTHOR, AND
BY OTHER WORKERS

Density of material tested	Conductivity as determined by			Author's representative value
	Finck	Finck and Van Dusen	A. S. H. V. E.	
<i>Bagasse</i>				
4.0	0.277	—	—	0.290
4.8	0.285	—	—	0.295
6.7	0.296	—	—	0.307
7.4	0.315	—	—	0.311
8.9	0.337	—	—	0.320
12.1	0.375	—	—	0.343
12.2	—	0.325	—	0.344
13.2	—	—	0.340	0.350
13.5	—	—	0.337	0.351
14.2	0.403	0.358	—	0.356
14.8	—	0.342	—	0.359
<i>Wood pulp</i>				
3.0	0.304	—	—	0.283
4.9	0.299	—	—	0.295
6.6	0.309	—	—	0.306
9.0	0.318	—	—	0.320
<i>Insulite</i>				
16.2	—	0.336	—	0.368
16.5	—	—	0.348	0.370
16.9	—	0.336	0.340	0.372

in the case of wood pulp is satisfactory, but at higher densities the agreement with bagasse is decidedly poor. However, when the results in the American Society of Heating and Ventilating Engineers' Guide Book (2) as well as the results in a paper by Finck and Van Dusen (7) are taken into consideration, it is found that the writer's mean values are between the values given in these two references and Finck's values as given in the first column in Table IV—provided of course due regard be paid to density. Moisture content has been assumed to be zero in these results, and where necessary the conductivity has been corrected to apply to a test carried out at a mean temperature of 90°F.

Conclusions

Apparatus

1. In view of the fact that moisture can migrate during a test from the hot side of the sample to the cold side, the hot-plate is not an ideal apparatus for testing the thermal conductivity of fibre wall boards in the commercial or undried state.

2. If wall boards are dried before testing, fairly consistent results can be obtained on the hot-plate apparatus. By improving the experimental equipment, advances in the accuracy of the test might be made, as for instance by the installation of an automatic control to maintain the current constant to 1/1000 of an ampere, and by arranging for a very accurate thermostatic control on the brine tank.

Data

1. It has been definitely proved that tests carried out on homogeneous fibre boards are of little value unless the moisture content is stated. Tests on samples containing moisture are inclined to be inaccurate even if the moisture content is stated, because there is a gradual migration of moisture from the hot side to the cold side. The result of this migration phenomenon is virtually the same as the effect of a very slow drying oven. Therefore, tests on dry sheets only should be considered as accurate. Tests on undried sheets have a certain value if both the moisture content and the duration of test be stated.

2. When the moisture contents of two fibre boards of the same thickness are equal, the really important factor in establishing their relative merits as insulators is density. It is unwise to compare the product of one manufacturer with that of another without taking into account the densities of the two products and ascertaining if these densities are typical of the manufacturer's product in that particular quality and nominal thickness of material. Results lead to the conclusion that the conductivity of fibre board between the densities of 3 and 18 lb. per cu. ft. is given by a linear band on a density-conductivity diagram.

3. Peculiarities in the structure of boards affect the thermal conductivity and therefore the points on the density-conductivity diagram do not lie accurately on a straight line but on a band. The differences between the conductivity values for different thicknesses and makes of wood-fibre board are as great as, or greater than, the differences between values for the wood-

and bagasse-fibre boards. When all factors, including density, are taken into account, bagasse and the two kinds of wood-fibre board tested by the writer did not differ much in insulating qualities.

4. Wood fibre in the loose state has been tested for thermal conductivity at a density as low as 3 lb. per cu. ft. and down to that density no evidence has been found to substantiate the belief that the thermal conductivity of fibre board increases with decrease of density.

5. A variation of 10°F. in the mean temperature of the sample appears to be of little importance in testing homogeneous fibre boards. Only with large changes of mean temperature does the change in thermal conductivity make its appearance. The magnitude of this change for fibre board has been determined and agrees fairly well with the work of other investigators.

Note.

Since this paper was written, a report has been found by the writer giving the results of some recent work by Cammerer on the effect of moisture content on the thermal conductivity of brick. This report appears on page 66 of "Heating and Ventilating", for April, 1932. Dr. Cammerer has found the influence of moisture content to be even more important in the determination of the thermal conductivity of brick than the writer has found it to be in the case of fibre board.

In the same issue of "Heating and Ventilating" there appears an article by Berestneff entitled "Moisture—its influence on the heat conductivity of building materials". Berestneff, after taking full account of Dr. Cammerer's work, remarks in summing up, "that all tables for coefficients of conductivity should also indicate the moisture content at which the values given in the tables are found." In the conclusion of the present communication the first sentence under "Data" expresses, as follows, almost exactly the same idea, "that tests carried out on homogeneous fibre boards are of little value unless the moisture content is stated."

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A NEW SWEEP-CIRCUIT DEVICE FOR THE CATHODE-RAY OSCILLOGRAPH¹

BY GEO. S. FIELD²

Abstract

On account of the frequency limitation of the ordinary sweep circuit used with the low-voltage cathode-ray oscillograph tube, a new circuit was developed for use in the analysis of higher-frequency alternating voltages and currents. This circuit has been employed to generate saw-tooth waves at frequencies up to 50,000 cycles per sec., and can probably be made to work at higher frequencies if necessary.

Introduction

The circuit (1) commonly employed for a time-base in connection with cathode-ray oscillography involves a discharge tube such as a neon glow tube or mercury-vapor thyratron. In such a circuit, a battery, high resistance and condenser are connected in series, while the discharge tube is connected in parallel with the condenser. Voltage gradually builds up across the condenser, until it reaches the break-down value for the tube. The condenser immediately discharges, the tube ceases to glow, and the process repeats itself. The result is a saw-tooth wave of voltage across the condenser, and it is this voltage which is connected to the oscillograph deflection plates.

This circuit works very well up to possibly 10,000 cycles per sec., but at higher frequencies difficulties arise on account of the time required for ionization and de-ionization of the gas in the discharge tube. The time-base described in this paper was developed to be independent of a gaseous discharge, and has not therefore the same frequency limitation.

The Circuit Used

In Fig. 1 the circuit is shown schematically. Tubes *A*, *B*, *G* and *C* are ordinary receiving valves, such as the UY-227. The diode *D* may be a 230 or 199 with the grid and plate connected together. Resistance r_1 has a value of 25,000 ohms; r_2 and r_3 are each of 50,000 ohms. V is 200 volts. The terminals T_1 are connected to the voltage whose wave-form is being analyzed, and the terminals T_2 go to the oscillograph. The action of the circuit is as follows.

During the positive half of a cycle of the voltage at T_1 , the grid of *A* is positive and *A* will therefore pass electrons from filament to plate. Tube *B* will pass current if its grid voltage is not too greatly negative, and this voltage is so adjusted that when c_1 is discharged and no current is flowing through r_1 , the grid has zero potential. Current accordingly flows through *B*, and causes a potential drop through r_1 as voltage builds up across c_1 . With c_1 charging, the voltage across r_1 is amplified by *G* and *C* to produce a slightly positive potential on the grid of *B*, which causes more current to flow through *B*. Voltage V , or a large fraction of it, now appears across c_1 .

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Contribution from the National Research Laboratories, Ottawa. This paper was read at the meeting of the Royal Society of Canada, May 1932.

² Junior Research Physicist, National Research Council, Ottawa.

This condenser then begins to discharge through D , and the potential drop across r_1 is reversed. This drop is amplified as before to produce a high negative potential on the grid of B . Hence no current flows through B until c_1

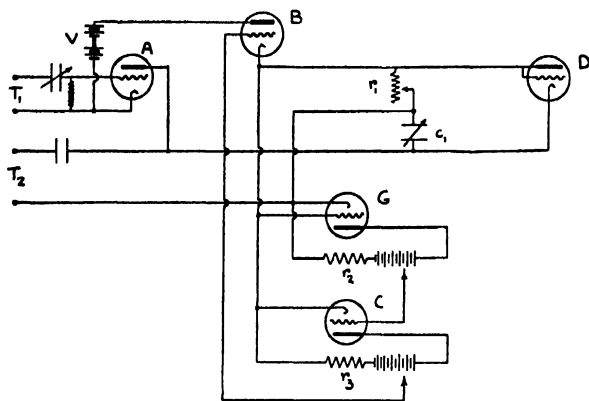


FIG. 1. Schematic diagram of sweep circuit.

the circuit in order that the saw-tooth wave shall be synchronized with the wave being analyzed. It is found in practice, however, that in most cases sufficient synchronization is obtained by connecting one wire from T_1 to a wire from T_2 , so that tube A may be taken out and the plate and filament terminals directly connected. The circuit is then made up simply of tube B , through which c_1 charges, tube D , through which it discharges, and the amplifier Gr_2Cr_3 which controls tube B .

Operation of Circuit

As an example of the results obtained by the use of this device in connection with the Von Ardenne oscillograph tube, Plate I is shown. The first figures in each case give the frequency of the wave under examination, and the second the time of photographic exposure. The two photographs taken at 43,200 cycles are included to show that the number of waves in a single sweep may be altered at will by controlling the time of discharge of the condenser. This is accomplished by altering the value of c_1 or changing the filament current of D .

The return sweep of the cathode-ray spot becomes more noticeable at the higher frequencies, because the time required for c_1 to charge is equal to one or two periods of the wave being examined. This could be improved by a more careful design of the amplifier, as at high frequencies the one used became rather inefficient. For this reason, in order to make B sufficiently negative during the discharge of c_1 , the bias on the grid of B had to be made more negative while c_1 was charging, and therefore B did not pass sufficient current to charge c_1 quickly enough.

Reference

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500 PER SEC., EXP = 11 SEC



43,200 PER SEC., EXP = 10 SEC.



43,200 PER SEC., EXP = 10 SEC.



102,000 PER SEC., EXP = 16 SEC.



149,000 PER SEC., EXP = 16 SEC



213,000 PER SEC., EXP = 14 SEC.

Example of figures obtained at various frequencies.

A NEW METHOD OF PREPARING COLLOIDAL SILVER AND GOLD BY MEANS OF A CONTINUOUS HIGH FREQUENCY ELECTRICAL DISCHARGE¹

BY A. N. FRASER² AND J. GIBBARD³

Abstract

A new method of preparing metal sols, by the use of a continuous high frequency electrical discharge of sufficient intensity to produce a sinusoidal spark, is described. Stable yellow colloidal silver and purple colloidal gold have been prepared, without a protective colloid, by this method. Chemical determinations have failed to indicate any silver oxide in the yellow silver sols.

A new method of preparing colloidal silver and gold by electrical dispersion has been developed. Bredig (1898) was the first to describe a method of producing colloids by electrical dispersion. Since that time other methods have been developed with the object of producing purer sols. Svedberg (3) in his new book, "Colloid Chemistry", thoroughly discusses this field. Most of these older methods give rise to colloids containing a great variation in particle size and also produce more or less marked changes in the dispersion medium. Thus far, the most satisfactory results have been obtained by using a high frequency spark.

High frequency undamped oscillations have been studied extensively, with considerable success, by Kraemer and Svedberg (2) and fully described by Svedberg (3). The high frequency undamped spark was produced by using a high frequency transformer in conjunction with a low frequency alternating current transformer. Since the oscillations from this type of circuit are neither continuous nor uniform, it seemed advisable to utilize the more modern methods of producing continuous, undamped, high frequency oscillations of sufficient intensity to produce a sinusoidal spark.

The following method was found to work extremely well. The high frequency potential required to maintain a discharge between electrodes immersed in redistilled water is induced into the discharge circuit by a driving circuit, employing a thermionic tube to produce the necessary high frequency sinusoidal current. The oscillator consisted of a Northern Electric type R-212 tube connected in accordance with the well-known Hartley arrangement. The high tension direct current supplied for the anode and the low tension direct current for heating the filament were obtained from motor generators. Where suitable generators are not available, alternating current might equally well be utilized by providing suitable transformers and a rectifier employing high efficiency mercury-vapor tubes.

Fig. 1 is a schematic diagram of connections to indicate the arrangement and characteristics of the circuit.

¹ Manuscript received July 26, 1932.

Contribution from the Radio Branch, Marine Department, Ottawa, Canada, and the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa.

² Senior Radio Electrical Engineer, Radio Branch, Marine Department, Ottawa, Canada.

³ Bacteriologist in the Laboratory of Hygiene, Department of Pensions and National Health, Ottawa, Canada.

It should be stated that the parts used in this oscillator were not specially made for the purpose, but were assembled from equipment available at the Test Room, maintained by the Radio Branch of the Marine Department, Ottawa.

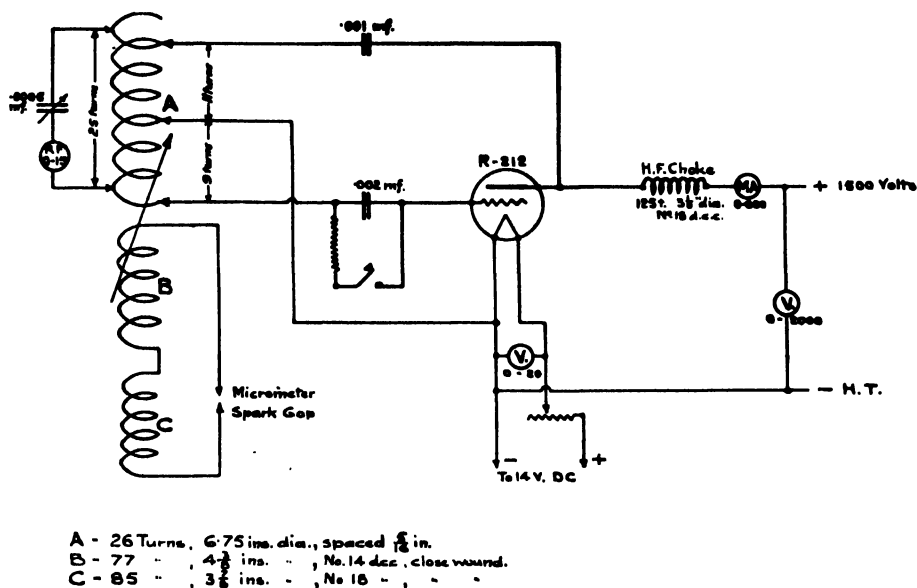


FIG. 1. Schematic diagram of high frequency continuous wave generator.

Coil *B* was inserted wholly within coil *A* in order to obtain sufficient transfer of energy to produce a discharge across the submerged gap. Coil *C* was added to bring the natural period of the discharge or secondary circuit into the range of frequencies obtainable in the driving circuit, by means of the variable condenser connected in parallel with the primary inductance.

The oscillator is first adjusted for stable operation at maximum output by varying the position of the grid and anode taps on coil *A* while noting the reading of the thermoammeter connected in series with the variable condenser. The discharge circuit is then adjusted with the gap in air until a discharge is maintained across the electrodes while they are at least 5 mm. apart. The electrodes are then adjusted to a gap of from 1 to 2 mm., immersed in a beaker of redistilled water and the oscillator energized. A continuous non-explosive discharge now takes place between the electrodes.

Within a relatively few moments after sparking, the dispersed metal may be readily seen forming a layer in the upper part of the distilled water above the electrodes. If sparking is carried on too long the water becomes very hot and the color of the sol changes. It is, therefore, advisable to use an ice bath to maintain a low temperature.

The size of particles seems to depend upon the nature of the spark, type of discharge electrodes, temperature of the water, hydrogen ion concentration, and many other factors. So far as the electrodes are concerned, it was found

that the most satisfactory results were obtained with knife edges about 5 mm. long. By this means, the size of the spark remains more uniform, since there is merely a change of its position at the knife edges, instead of a change in length as the metal is dispersed. When flat-faced electrodes are used, a bubble of gas collects between them, and then, instead of having the spark pass through the water, it passes through the gas, with the result that the metal is dispersed in larger particles. If, on the other hand, fine wire electrodes are used, the length of the gap increases as the metal is dispersed or melted, giving unsatisfactory results.

Colloidal Silver

By this method, stable yellow colloidal silver can be readily prepared in pure redistilled water, without the addition of any protective colloid. By varying the experimental conditions, black, green, greenish-yellow, and orange-yellow silver sols have been prepared.

The quantity of silver dispersed has been measured in two instances. A black sol contained 16 mg. of silver per litre, while a bright yellow sol contained 2 mg. per litre. Undoubtedly more concentrated sols could be prepared, especially if a method could be developed to continuously remove the more concentrated upper layers.

As a test for the purity of silver sols, hydrogen sulphide was bubbled through a yellow silver sol and a solution of silver nitrate, each containing 1:100,000 silver. There was an immediate distinct darkening of the silver nitrate, but no change in the colloidal silver. However, it was noticed that within an hour or so the color of the silver sol was considerably reduced. It is considered significant, in the light of Kohlschütter's (1) statement that silver sols prepared by Bredig's method contain 19-52% of silver oxide, that there was no darkening of this silver sol. Silver oxide may be produced by this method of sparking, but it was not detectable as a sulphide. As a further test for the presence of silver oxide, the reaction described by Whitby (4) was applied. The author claims that this method will detect one part of silver in 25,000,000 parts of solution; preliminary determinations indicated that such is the case. When Whitby's method was used, silver could not be detected in these silver sols unless the sol was first treated with nitric acid. Conductivity determinations would probably settle this question, but unfortunately the necessary apparatus was not available.

Colloidal Gold

Red colloidal gold with a yellowish sheen has also been prepared, but thus far, only in the presence of a minute amount of gelatine as a protective colloid. In pure, freshly redistilled water, a purplish-red gold sol has been prepared and thus far appears to be quite stable. It would seem that it is mainly a question of solving technical details to prepare a red gold sol.

Conclusion

It has been found possible to prepare stable yellow silver and purplish-red gold sols in redistilled water without the presence of any protective colloid.

It would seem, therefore, that a continuous high frequency electrical discharge offers some advantages in the preparation of metal sols. Further work is in progress and will be reported in later publications.

Acknowledgment

The authors gratefully acknowledge the courtesy and assistance given by Commander C. P. Edwards of the Marine Department and Mr. E. D. Hayman of the Test Room, Radio Branch, Marine Department, Ottawa.

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THE DISTRIBUTION OF IODINE IN ALBERTA IN RELATION TO THE PREVALENCE OF GOITRE

I. IODINE IN THE WATER SUPPLIES¹

BY OSMAN JAMES WALKER²

Abstract

A preliminary study of the iodine content of the water supplies of the Province of Alberta has been made. The iodine content of the surface water is usually less than one part of iodine per billion (1000 million) parts of water. The iodine content of water from deep wells varies from 0 to 660 parts of iodine per billion parts of water. There is a relation between the iodine content of deep wells and the geological strata from which the water is derived. A goitre survey of the province is in progress and a map shows its distribution. A close relation between the iodine content of the water and the prevalence of goitre has not been revealed so far. A number of factors that may affect this relation have been discussed.

During the last ten years many investigations have been undertaken on the occurrence of iodine in waters, foods, soils and rocks. Attempts have been made to show that the prevalence of endemic goitre in a district (6, 8, 11, 14, 18, 23) is inversely proportional to the intake of iodine in water and foods. In the iodine surveys of the waters of Switzerland (5), New Zealand (12), and various parts of the United States (4, 10, 19, 24), it has been found that there is a reasonable correlation between the lack of iodine in the water and goitre.

Iodine in the Waters of Alberta

Since no systematic survey on the distribution of iodine has been made in Canada, it was decided to begin work in Alberta. It was considered advisable to start on water supplies with the intention of examining foods, soils and rocks later. At the same time information on goitre was assembled.

Since the iodine content of water is low, large samples for analysis are required. It was decided that 100 litres or 25 gal. would be the amount that would give most satisfactory results. Arrangements were made with doctors, teachers, agriculturalists, engineers and university students residing at various places in the province to obtain the samples, to evaporate them down to a volume of about a litre and to ship the prepared samples by express to the university. During the evaporation, the samples were kept alkaline by sodium carbonate and any crystals which formed were discarded. Data were obtained on the sources of samples and also information on the occurrence of goitre in the neighborhood. Table I shows the form of questions asked.

The method which was used for analysis of these samples is based on that proposed by McClendon (20, 22) and his coworkers, with a few minor modifications. The water was evaporated to dryness on a water bath. In some of the samples, where the dissolved solids were high, it was necessary to filter off and discard the crystals as they formed. A number of these residues were

¹ Manuscript received June 20, 1932.

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² Associate Professor of Chemistry, University of Alberta.

TABLE I

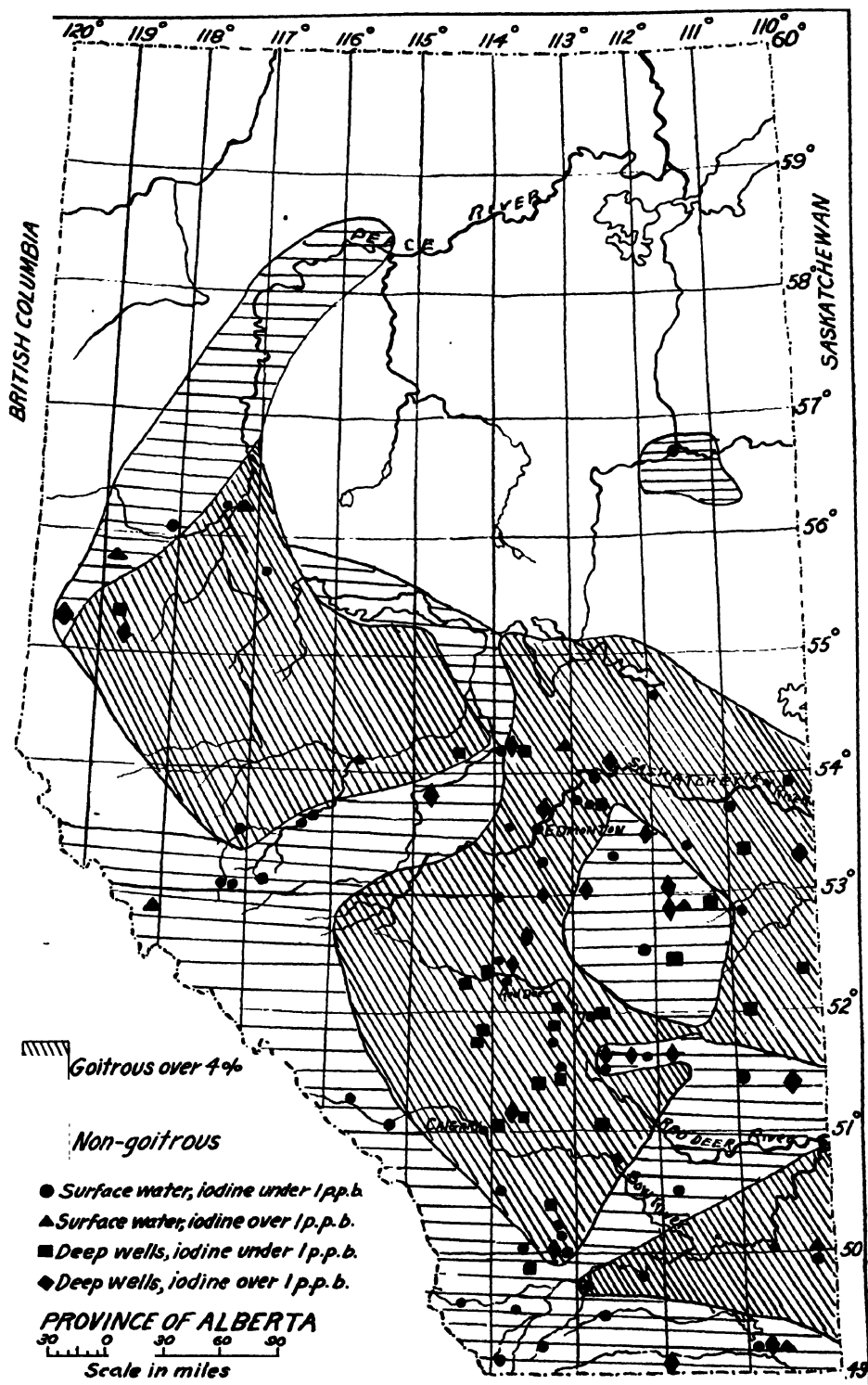
-
-
1. Dates of collecting sample.....
 2. Total volume of water used.....
 3. Source of sample (river, well, etc.).....
 4. If well or spring state depth and whether water comes from sand, sandstone, shale, limestone or clay.
.....
 5. Ordinary use of water supply (household, animal, community, industrial, etc.)
.....
 6. Can you give me any opinion on the prevalence of goitre, both in humans and animals in your neighborhood?
.....
.....

Sec.....Tp.....Range.

Address.....

Name.....

tested and it was found that their iodine content was negligible. The next step was to destroy all organic matter by heating in a muffle furnace at 450 to 500° C. for five hours. The calcine was then ground up and extracted with 95% alcohol. This removed the bulk of the iodine. The residue was then extracted with water and a separate determination on its iodine content was carried out. In many cases the extraction of iodine by alcohol was complete but in some aqueous extracts, especially when the iodine content of the water was high, appreciable amounts were found. The alcohol extract was evaporated to dryness, taken up with one or two cc. of water, made acid with orthophosphoric acid, and treated with 1 cc. of 0.1 *N* sodium sulphite to reduce any iodate to iodide, and transferred to a small separatory funnel. One cc. of sodium nitrite solution, 2 mg. per cc., was added and the volume made up to 10 cc. One cc. of c.p. carbon tetrachloride was then added. The contents of the funnel were shaken thoroughly and the carbon tetrachloride layer transferred to the cup of a Duboscq microcolorimeter and the color compared with a standard solution of iodine in carbon tetrachloride containing 0.1 mg. of iodine per cc. In the calculations it was assumed that the concentrations of iodine in the water layer (13) and the carbon tetrachloride were as 1:85. The water extract was evaporated to about 2 cc. and any crystals which appeared before or at this point were filtered off. The procedure from this point was the same as that used for the alcohol extract, except that it was sometimes necessary to increase the volume of water at the time of extraction with carbon tetrachloride owing to the high concentration of salts in the solution.



The total iodine was equal to the sum of the values obtained for the two extracts.

One hundred and eighteen samples of water from different parts of the province were examined, the iodine content ranging from less than 0.1 parts of iodine per billion parts of water up to as high as 660 parts per billion. Table II shows the values which were obtained.

Fig. 1 is a map of Alberta showing points from which samples were obtained.

In the classification an attempt has been made to designate wells deriving water from the glacial drift as "shallow" and those deriving water from definite beds as "deep". For this reason, relatively shallow wells at Vegreville and Smoky Lake are classified as deep wells and, although fairly deep, the well at Empress is classified as a shallow well due to the thick layer of glacial drift at that point.

Referring to Table II it will be seen that surface waters, either from streams or from shallow wells, are low in iodine, containing as a rule, less than one part of iodine per billion parts of water. There are several exceptions to this rule. For example, the water from Jasper Park is high in iodine content. This is likely due to the fact that the water trickles through rocks of Precambrian age, which usually contain large amounts (2, 7) of iodine. So far it has not been possible to explain the high iodine values of surface waters from Peace River, Thorhild, Walsh and Manyberries.

The waters from deep wells are more variable in iodine content. They contain from 0 to 660 parts of iodine per billion parts of water. There is undoubtedly some connection between the iodine content of the water and that of the rock through which it passes. Fig. 2 is a geological map of the province (1), on which has been placed the location of the deep wells with their depth and the iodine content of the water. Deep wells in the area underlain

TABLE II
IODINE CONTENT OF WATER IN PARTS PER BILLION

Source	Parts per billion	Source	Parts per billion
Streams			
Banff (Bow)	0	Blairmore (Crows' Nest)	0.31
Sterco (Springs and mine water)	0	Edmonton (N. Saskatchewan)	0.33
Pigeon Lake (lake)	0	Red Deer (Red Deer)	0.35
Pincher Creek	0.1	Edson (McLeod)	0.35
Medicine Hat (S. Saskatchewan)	0.1	Gull Lake (lake)	0.38
Claresholm (Willow Creek)	0.1	Waterton (creek)	0.41
Calgary (Bow)	0.1	Evansburg (Pembina)	0.42
Cardston (creek)	0.1	Exshaw (Bow)	0.42
Fernie, B.C.	0.1	Cadomin (McLeod)	0.55
Blairmore (Crows' Nest)	0.1	Calgary (Bow)	0.64
Raymond (St. Mary)	0.12	Raymond (St. Mary)	0.96
Lethbridge (Old Man)	0.18	Taber (Old Man)	1.0
McLennan (Athabasca)	0.25	Manyberries (run-off water)	3.95
Brooks (Bow)	0.27	*Lethbridge (Old Man)	4
Bassano (Bow)	0.29	†Manyberries (reservoir)	5.2
Edmonton (N. Saskatchewan)	0.25	Jasper Park (lake)	21

TABLE II—Continued

Location	Depth, ft.	Parts per billion	Location	Depth, ft.	Parts per billion
Shallow wells and springs					
Champion	30	0	Craigmyle	20	0.23
Innisfree	50	0	Carbon	25	0.3
Fort Saskatchewan	22	0	Forestburg	25	0.32
High River	30	0	Manyberries	70	0.34
Three Hills	14	0	Whitcourt	25	0.46
Barons	6	0	Cadomin (spring)		0.5
Brierville	20	0	Bruderheim	20	0.6
Leduc	20	0	Fairview (shallow)		0.6
Fort McMurray	46	0	Big Valley	25	0.61
Chinook	30	0	Spruce Grove (spring)		0.66
Ryley	10	0	Empress	80	0.72
Lamont	54	0.02	Heinsville	36	0.73
Wainwright	30	0.04	Westlock	30	0.78
Carmangay (spring)		0.1	Killam (shallow)		1.77
Slawa	20	0.1	Spirit River	18	1.8
Marlboro	25	0.1	Thorhild	20	2.4
Grimshaw	60	0.1	Walsh (springs)		4.53
Delph	20	0.16	Peace River	13	32.8
Walsh	18	0.21			
Deep wells					
Elnora	150	0	Conrich	147	2.1
Olds	260	0	Lacombe	240	2.25
Standard	75	0	Ponoka	204	2.58
Vulcan	200	0	Michichi	80	2.8
Sexsmith	108	0.1	Rochfort Bridge	98	3.0
Consort	74	0.1	Conrich	127	6.5
Granum	100	0.11	Coutts	80	6.85
Ogden	176	0.12	Hanna	110	7.3
Blackfalds	75	0.32	Smoky Lake	50	7.6
Bircham	250	0.38	Westlock	125	10.37
Didsbury	160	0.41	Hythe	100	23.0
Barrhead	325	0.45	Vegreville (wells)	50-72	25.1
Big Valley	175	0.56	Lloydminster	180	25.6
Alliance	85	0.68	Camrose (wells)	130-260	26.2
Irma	84	0.74	Viking	100	29.8
Provost	170	0.76	Oliver	250	30.6
Huxley	180	0.8	Grande Prairie	400	81.0
Eckville	200	0.83	Wetaskiwin	300	102.9
Vermilion	125	1.0	Barons	232	106.6
Bruderheim	135	1.08	Wetaskiwin (wells)	272-320	164.3
Clyde	200	1.1	Manyberries	1136	222.8
Conrich	74	1.4	Killam	300	436.0
Carbon	110	1.6	Killam	243	631.6
Oyen	500	1.85	Killam	260	663

*Evaporated in a schoolroom where iodine had been prepared.

†A reservoir contaminated by water from a deep well rich in iodine.

by rocks of the Paskapoo series of Tertiary age yield water which is low in iodine, usually containing less than one part per billion. An apparent exception to this rule was found in the case of the water at Wetaskiwin, but a more recent geological survey (25) definitely places Wetaskiwin, and possibly Ponoka, in the territory underlain by rocks of the Edmonton series. Deep-

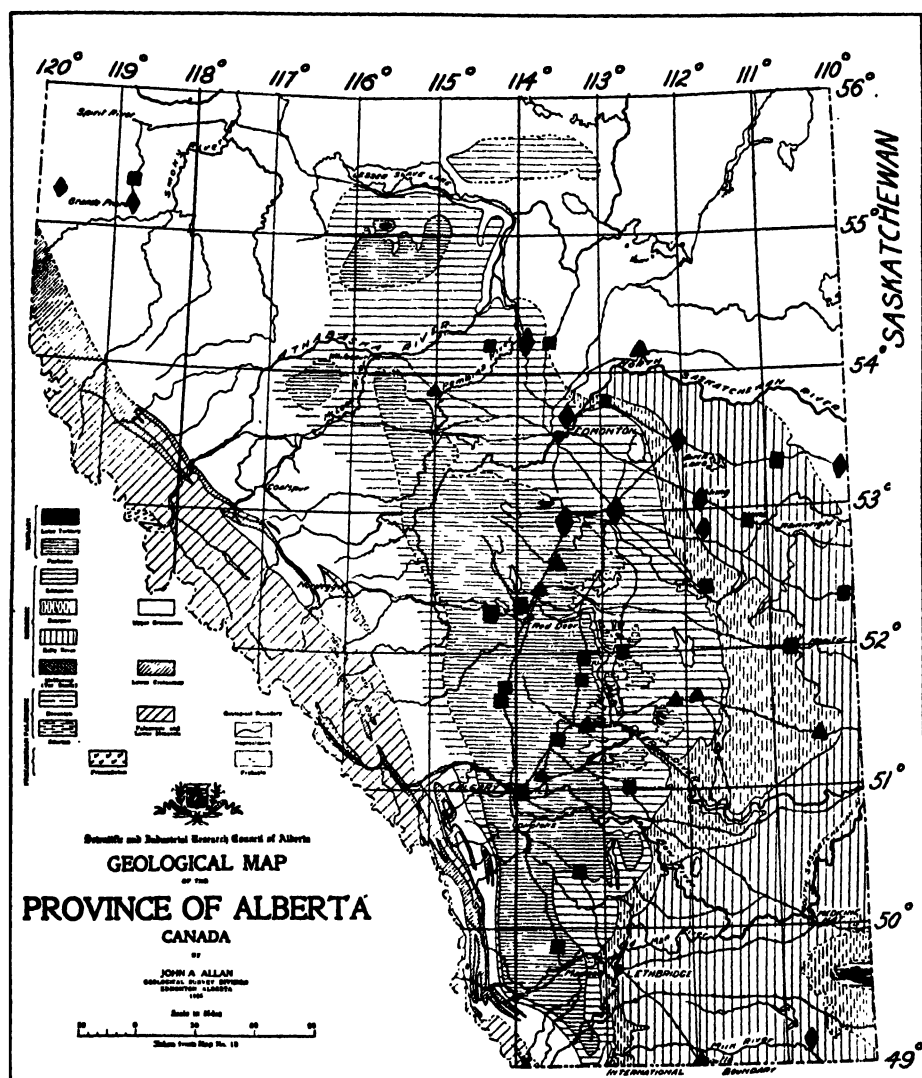


FIG. 2. Geological map of Alberta showing the location of the deep wells and the iodine content of the water. ■ Deep well water; iodine, 0-1 p.p.b. ▲ Deep well water; iodine, 1-10 p.p.b. ♦ Deep well water; iodine, over 10 p.p.b.

well waters derived from the rocks of the Upper Cretaceous, especially in the Edmonton and Belly River Series, are generally rich in iodine. Low iodine values were obtained for water from wells in the Bear Paw Series, the rocks of which are older than those of the Edmonton Series but younger than those of the Belly River Series. The highest iodine values were found in the waters of three wells north of Killam in the Belly River Series. These wells were 243, 260 and 306 feet in depth, and the iodine contents of the waters were 631.6, 663 and 436 parts of iodine per billion parts of water respectively. Other iodine values of 100 and more parts per billion were found in waters

TABLE III
PREVALENCE OF GOITRE AS REPORTED BY COLLABORATORS

Edmonton	Goitre in both humans and animals.
Barrhead	There are a considerable number of cases of goitre in the district.
Lethbridge	One hundred and one cases in Galt Hospital in five years. So goitre seems to be rather common.
Taber	More or less in the goitre belt. Goitre not uncommon.
Consort	Uncommon.
Lamont	Very little.
Sexsmith	A considerable amount among people.
Wainwright	Average in humans.
Walsh	Knows of no goitre.
Alliance	Rare.
Vermilion	In animals on school farm.
Viking	Very little goitre (well 70-100 ft.)
Cadomin	None known.
Champion	Quite prevalent in humans and animals.
Forestburg	Not many cases among humans.
Olds	"About normal." Worse in wet years than in dry years. Quite prevalent in livestock, but sporadic. Some families contain as many as five suffering from goitre.
Whitecourt	Prevalent in calves and colts. Human goitre not noticeable.
Irma	Not prevalent in either humans or animals.
Craigmyle	Six or seven cases in humans in last few years. Knows of none in animals.
Provost	Probably less than 1%.
Ogden	Some cases.
High River	Fairly prevalent in humans and animals.
Hanna	Not very prevalent.
Oliver	Not common in humans nor in animals.
Delph	Occurs occasionally in humans and animals. At least one case of goitre on farm from which the sample of water was obtained.
Pincher Creek	Some goitre in both humans and animals.
Spruce Grove	Some in calves, colts and pigs at birth. Does not believe it is prevalent in humans.
Killam	No goitre in animals in district. Some in people.
Three Hills	About 1%.
Calgary	Prevalent.
Wetaskiwin	Six cases in last six months in about half the population of the town.
Clyde	None in humans and animals.
Carmangay	Very little in animals and humans.
Ponoka	Some in humans and animals. Not a great deal.
Medicine Hat	Not very prevalent.
Barons	Five or six cases in women in radius of 10-15 miles. Some in livestock.
Barons	Some in humans.
Manyberries	No known cases.
Slawa	Apparently rare.
Smoky Lake	Not prevalent in animals. Considerable adolescent goitre in humans.
Brierville	Rare.
Westlock	Moderate—about 10% in humans.
Vegreville	Very little in humans and animals.
Claresholm	Rare.
Blairmore	Not prevalent in humans. Some in cattle.
Rochfort Bridge	Occasionally appearing in calves and lambs.
Bruderheim	Knows of five cases in humans.
Westlock	Common among sheep.
Gull Lake	Quite prevalent.
Lacombe	Quite prevalent.
Sterco	None.
Marlboro	50%—high.
Wetaskiwin	Quite prevalent.
Calgary	Fairly common among young people.
Exshaw	No cases.
Edson	Knows of no cases.
Pigeon Lake	No goitre among humans.
Brooks	Not unduly prevalent.

TABLE III—*Continued*

Waterton Lakes	A few cases in humans. No cases in animals noticeable.
Leduc	Fairly prevalent in humans.
Thorhild	None in this district. Some in humans in neighboring school district.
Grimshaw	Ten per cent or higher in humans. Quite prevalent in animals.
Coutts	No cases in children and animals known. Some in adults of middle age.
Peace River	Fairly prevalent, especially along inland waterways.
Eckville	Prevalent among humans, rare among animals.
Grande Prairie	Not common in the town.
Fairview	Very little among humans. Prevalent among animals.
Cardston	Quite prevalent among humans. Unable to state about animals.
Michichi	Not prevalent. Only a few cases in the district.
Heinsville	Knows of six cases in humans—probably more.
Jasper	Not prevalent.
Big Valley	Prevalent. Several cases in town.
Oyen	Rather rare in lambs and calves. Fifteen cases in Cereal in eight years.
Didsbury	Very prevalent among children.
Fernie	Fairly common, especially among older people.
Standard	Two cases known within eight miles.
Raymond	Rare (previously, water taken from deep well).
Huxley	Several cases in the neighborhood (two on this farm).
Spirit River	Very rare. Eleven out of forty children in a school nearby have the disease.
Fort McMurray	None.
Conrich	None within four miles.
Granum	Some in the district.
Evansburg	Ten cases in four years.
Blairmore	None in humans and very little in animals.
Chinook	Not prevalent.
Hythe	Not common.
Killam	None on farms with deep wells.
Red Deer	Thirty-two cases in the town schools (549 students, 5.8%). Quite prevalent in animals.
Bentley	Seven cases in 98 school children = 7%.
Bowden	Sixteen cases in 109 school children (14 being girls) = 15%.
McLennan	Some.

from wells at Wetaskiwin and Barons in the Edmonton Series and also at Manyberries in the Belly River Series. Values between 25 and 100 parts per billion were found in water from wells at Viking and Vegreville in the Belly River Series, Oliver and Camrose in the Edmonton Series, and Grande Prairie and Lloydminster in the undivided series in the Upper Cretaceous. The deepest well from which a sample was tested has a depth of 1136 ft. and is located near Manyberries. This is an artesian well owned by the Dominion Range Experimental Station in the southeastern corner of the province.

As previously mentioned, three deep wells were sampled near Killam. Samples of water were also obtained from three deep wells in the Conrich district northeast of Calgary. The iodine contents of the three samples were similar to one another although the values were all higher than expected from their geological position.

It will be noted in Table III and Fig. 1 that a number of samples were obtained from the same locality but were derived from dissimilar sources. The water from a river and a spring at Cadomin contained almost identical amounts of iodine. Surface and near-surface waters from the vicinity of Manyberries, Bruderheim, Barons, Westlock and Killam were much lower in iodine content than the water from the deep wells in the same district. In

many other districts there are also varied sources of water supply but these have not been investigated as yet.

By referring to the maps in Figs. 1 and 2 it will be seen that the waters of some districts have not been tested and some need a closer examination. It has been difficult to find collaborators for collecting and preparing the samples in districts which are sparsely settled and isolated from the centres of population. It is hoped that the survey may be further advanced in the near future.

Goitre Survey

Nearly every collector of the water samples for the iodine test furnished information on the prevalence of goitre in their districts. In many cases they consulted with the local physicians and veterinarians so that the data are fairly reliable. The information so obtained is presented in Table III.

Valuable information was also obtained from the Travelling Health Clinics which were sent to various rural centres in the province by the Department of Health during the summers of 1929, 1930 and 1931. Data for 1931 are not yet available. Table IV shows the information on goitre collected by the clinics during 1929 and 1930. The health clinics operated as follows. A centre was chosen and the nurses examined the children in the schools in the neighborhood. Those who required any medical or dental attention were asked to report at the clinic. Each clinical centre represented about 12 school districts. Column 2 shows the number of children examined by the physicians at the centre; column 3 shows the number examined by the nurses in the neighboring schools; column 4 contains the number of children having thyroid enlargements; and column 5 is the percentage of goitre in comparison to the total number examined by the nurses. It will be noted that for some of the centres no statistics are given regarding the number of children examined by the nurses. These statistics are lacking because of either no examination in the schools, or because of independent examinations by district nurses or district physicians.

The percentage of goitre in children as shown in this table varies from zero to 22.5%.

Additional information has been obtained from doctors and veterinarians in various parts of the province.

By using the information from these sources, a tentative goitre map for Alberta has been compiled and appears along with the source of water samples on Fig. 1. On this map, goitrous districts represent an incidence of over 4% as indicated by the data available. It is difficult to draw definite boundaries between goitrous and non goitrous areas because of the fact that often a small district where goitre is prevalent may be surrounded by districts where thyroid enlargement is rare and *vice versa*. There is little evidence of goitre in the mountain region among humans although there is some among animals. Goitre is not prevalent near the southern boundary of the province. There is a great deal of goitre in a strip of territory between Edmonton and Calgary. Goitre is prevalent in the irrigated districts of the southern part of the province and also in the north in part of the Peace River territory where drinking

water comes chiefly from rain and snow. Some of the newly settled districts not yet reached or just recently served by railways are more goitrous than those in the same vicinity but settled for a longer time.

TABLE IV
NUMBER OF THYROID CASES AMONG CHILDREN PRESCRIBED FOR BY
THE ALBERTA TRAVELLING CLINIC

Clinic centre	No. of children examined by physicians	No. of children examined by nurses	No. with goitre	% with goitre	Clinic centre	No. of children examined by physicians	No. of children examined by nurses	No. with goitre	% with goitre
1929									
Pincher Creek	97	223	5	2.2	Kinuso	57	69	3	4.3
Dunstable	43		3	7	Strathmore	154	202	24	11.9
Coutts	99	218	4	1.8	Bulwark	75	307	4	1.3
Markerville	104	194	13	6.7	Hillspring	257	307	5	1.6
Cessford	158	164	2	1.2	Tawatinaw	86	204	11	5.4
Jarvie	80	89	2	2.2	Plamondon	164		11	
Grouard	91	196	4	2	Lac la Biche	127	346	10	6.1
Millet	152	188	14	7.4	Breton	52	137	8	5.8
Sundre	79	205	13	6.3	Oyen	80	180	4	2.2
Diamond City	197	390	11	2.8	Pendryl	68	78	8	10.3
Naco	101	98	3	3	Slave Lake	38	69	0	0
Youngstown	49	189	2	1.1	Wanham	72		5	7.0
Collholme	79	221	4	1.8	Clyde	100	211	6	2.8
New Bridgen	149	218	7	3.2	Sangudo	100	298	2	.6
Didsbury	112	188	26	13.8	Mountain View	101	240	1	.4
Mooside	83	130	5	3.8	Onoway	154	270	6	2.2
Wabamun	99		2	2	Mirror	200	386	8	2.1
Darwell	78	123	4	3.3	Clive	213	359	15	4.2
Mayerthorpe	154	137	8	5.8	Eastburg	44		1	2.3
Cherhill	89	149	3	2					
1930									
Mannville	226	357	39	10.9	Rumsey	144	130	11	8.5
Paradise Valley	126	306	19	6.2	Irvine	80	154	11	7.1
Metiskow	175	191	20	9.5	Whitla	64	80	6	7.5
Lakesend	120	120	27	22.5	Orion	119	135	10	7.7
Henaruka	155	150	10	6.7	Wrentham	60	249	3	1.2
Altario	140	268	10	3.7	Del Bonita	64	89	2	2.2
Sibbald	159	375	7	1.9	Travers	147	266	5	1.9
Sunnydale	124	195	5	2.6	Fort Vermilion	155		4	2.6
Sunnynook	115	79	9	11.4	Notikewin	127		4	3.3
Peers	65	100	2	2	Grimshaw	82		8	9.8
Strome	107		11		Whitelaw	138		5	3.7
Killam	97	610	11	3.6	Spirit River	93		2	2.1
Halkirk	110	248	2	0.8	Valhalla	150		22	14.7
Byemoor	126	196	21	10.7	Beaver Lodge	153	154	12	7.8

Relation Between Iodine in the Water and Prevalence of Goitre

In correlating the prevalence of goitre in Alberta with the lack of iodine in the water supply, the relation does not hold as closely as has been found in other countries. For example, in the mountain region where the iodine content of the water is low, goitre is rare, but in the foothills and neighboring districts

to the east where the iodine content of the water is also low, goitre is endemic in both humans and animals.

Iodine as a prophylactic for goitre has been known since the early work of Chatin (3) and is now used to a very large extent in goitrous countries in the form of iodized salt. This is not only true in districts where iodine surveys have been made but also in other districts. Table V, for example, shows the amount of the different varieties of salt sold by a wholesaler in the Edmonton district.

TABLE V
AMOUNTS OF SALT SOLD BY A WHOLESALER IN
THE EDMONTON DISTRICT

	Non-iodized, lb.	Iodized, lb.
Salt in 50-lb. bricks	800,000	250,000
Salt in bags	1,600,000	
Salt in cartons	14,400	72,000

These amounts represent approximately one-tenth of the total consumption of salt for the province. Salt in bricks is used for animals while other forms of salt are for household and industrial uses. In some places on this continent (16, 26) the drinking water is periodically treated with sodium or potassium iodide and the amount of goitre has been thereby materially reduced.

The water supply of a region is obtained from many diverse sources. Even in a small area the water may be obtained from streams, or shallow wells, or from deep wells that tap strata at different depths below the surface. Water dissolves soluble salts from the soil or rock with which it comes in contact as it moves on the surface or underground. It has been shown (9, 15, 17) that soils and rocks contain variable amounts of iodine in combination. Part of this is leached out and enters the water. The amount of iodine entering the water will depend on the time the water has been in contact with the soil or rock, also on the richness of the soil or rock in iodine, and on its solubility under the conditions of leaching. For these reasons the iodine content of the water supply may be variable in one district. Plants take up varying amounts of iodine depending on the quantity of this element in the soil and the ground water. A district may obtain all of its water supply from deep wells containing a high iodine content (21) but the plants will be low in this element due to the fact that the surface water with which they come in contact is low in iodine. In some districts practically all of the food is brought in from outside sources. All of these factors must be kept in mind if one wishes to draw any relation between the amount of iodine and the prevalence of goitre.

The Province of Alberta has an area of about 260,000 square miles and contains a great variety of topographic features. The soil on the surface is largely composed of glacial drift. It is underlain by rocks of many ages, ranging from Precambrian to the later Tertiary. In the mountain districts most of the food is imported but on the plains a great deal is locally grown. Iodized salt, as shown, is used more or less extensively. Drinking water supplies are obtained from streams, shallow wells, deep wells and even from rain and snow. The population of Alberta consists of people of many nationalities who practise different methods of cooking. There will undoubt-

edly be varying amounts of iodine lost during the cooking processes. This factor has been discussed by Stiner (27) in attempting to correlate goitre with iodine in parts of Switzerland. With the variety of conditions prevailing in Alberta the difficulty which is met with in correlating iodine with goitre will be understood.

Further studies on the distribution of iodine in Alberta are being undertaken and it is hoped that more light will be shed on the relation of this element to the occurrence of goitre.

Acknowledgment

The author wishes to express his thanks to Dr. M. R. Bow, Deputy Minister of Health, who furnished the statistics gathered by the Travelling Clinics; to the members of the Geology Department of the University of Alberta who gave special details on geology; to the officials of the Provincial Laboratory who assisted in the transportation of the water samples; and to all of those persons who collected and prepared the water samples throughout the province.

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THE KINETICS OF THE OXIDATION OF GASEOUS ACETALDEHYDE¹

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Abstract

The kinetics of the oxidation of gaseous acetaldehyde have been investigated from 60° to 120° C. by observing the rate of pressure decrease in a system at constant volume. A considerable induction period exists, during which the main products of the reaction are carbon dioxide, water, and formic acid. The main reaction in the subsequent stages involves the formation of peroxides and their oxidation products. The heat of activation of the reaction is 8700 calories per gram molecule. The indications are that the reactions occurring during the induction period are heterogeneous. The subsequent reaction occurs by a chain mechanism. The chains are initiated at the walls of the reaction vessel, and are also largely broken at the walls.

Introduction

A kinetic study of the oxidation of many organic compounds in the gaseous state has led to the conclusion that these reactions occur by a chain mechanism. Under such conditions a large number of molecules react for each primary step which occurs, and hence the order of the reaction is abnormal and not necessarily integral, and the rate of reaction differs from that which might be expected from the heat of activation. Any factor which influences the propagation of the chains will have a pronounced effect on the rate of reaction, and hence such reactions are often influenced by foreign gases, inhibitors, and the walls of the container. In general, the characteristics of such a reaction will depend on the length of the chains, and on the manner in which they are initiated, continued, and broken.

Hinshelwood and his coworkers have investigated the oxidation of ethylene (15), benzene (9), methane (10), methyl alcohol (10), and formaldehyde (10). All these reactions proceed by a chain mechanism, and in each case an induction period occurs, which is explained as due to the formation of unstable peroxides which serve as centres from which chains are propagated.

It seems advisable to extend such investigations to the study of the oxidation of other organic compounds, and the present paper deals with the gas-phase oxidation of acetaldehyde. It is unnecessary at this point to refer to the extensive literature on the oxidation of acetaldehyde, but such details as have a direct bearing on this investigation will be mentioned in the appropriate place.

Apparatus

The reaction was investigated by introducing acetaldehyde-oxygen mixtures into a heated vessel and observing the rate of change of pressure as the reaction proceeded.

The apparatus employed was similar to that used by one of the authors for

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the oxidation of acetone (14), and is illustrated in Fig. 1. The reaction bulb, *A*, was contained in an electric furnace, *B*. The temperature was measured by a standard thermometer, *D*, and the furnace was hand-regulated by rheostats

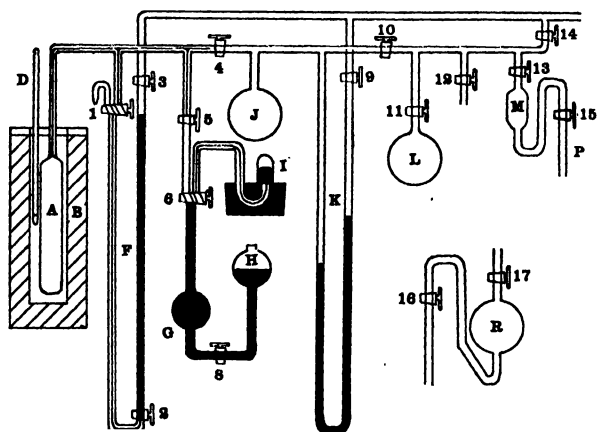


FIG. 1. Apparatus.

The necessity of this precaution has been shown in a previous publication (11).

Oxygen and acetaldehyde were stored in the containers *L* and *M*, and mixtures of the desired proportions were made by admitting these as gases to the mixing bulb, *J*, and observing their partial pressures by means of the manometer, *K*.

The oxygen employed was obtained from cylinders and was dried over phosphorus pentoxide. Acetaldehyde was prepared from a high grade of paraldehyde by distillation with a few drops of concentrated sulphuric acid. It was redistilled in the presence of carbon dioxide immediately before each experiment, and was drawn over directly from an ice-cooled receiver to the acetaldehyde reservoir through tube *P*. Thus the acetaldehyde used was in contact with oxygen only for a very short time before reaching the reaction bulb. The importance of this has also been pointed out in a previous communication.

The second reaction bulb, *R*, was used instead of the simple bulb for those experiments in which the condensable products of the reaction were investigated.

Experimental Procedure

Prior to each experiment the reaction bulb was evacuated for a few hours while kept at about 350°C. This was found to be necessary in order to remove material from the walls which affected the rate. The furnace was then brought to the desired temperature, and some of the gas mixture in *J* was admitted to the reaction bulb through tap 4, tap 1 having been previously closed. Tap 4 was then closed and tap 1 opened, and the initial pressure of the mixture was read on the capillary manometer. A small quantity of air at a pressure slightly greater than that of the mixture had been previously admitted to the manometer to serve as a buffer. Tap 1 was opened only momentarily for the pressure readings, which were made at regular intervals during the course

to within 1°C. The capillary tubing between the reaction bulb and taps 1, 4, and 5 was wound with nichrome wire and maintained at the temperature of the furnace.

The capillary manometer, *F*, was not heated. A small amount of air could be admitted to the manometer through tap 1. This served as a buffer to keep the mixture out of contact with the mercury surface.

of the reaction. The correction for capillary depression was made automatically by taking the zero reading of the manometer as the position of the mercury when the system was evacuated.

By means of the bulbs *G* and *II* samples of gas for analysis could be pumped out of the reaction vessel and collected over mercury in the tube *I*. For those experiments in which it was desired to obtain an aqueous solution of the condensable products for analysis, the reaction bulb *R* was substituted for *A*. When the reaction had reached the desired stage, the furnace was removed, tap 17 was closed, the exit tube was immersed in distilled water, and on opening tap 16 water was sucked into the reaction bulb. The solution thus obtained, together with four washings, was collected and made up to 500 cc.

The Products of the Reaction

The quantity of condensable products, including unchanged acetaldehyde, was estimated from the pressure change obtained on cooling the products in the reaction bulb to -78°C . While the reaction bulb was thus cooled, samples of gas were removed for analysis to a modified type of Bone-Newitt gas analysis apparatus.

The aqueous solution of the reaction products was tested quantitatively for the following: (a) total acidity with 0.02 *N* sodium hydroxide; (b) peracids and peroxides with neutral potassium iodide and acidified potassium iodide respectively, and subsequently with 0.02 *N* sodium thiosulphate by the method of Clover and Houghton (4); (c) total reducing agents with alkaline permanganate according to the method of Cameron and McEwan (3); (d) a portion of the solution was heated for from four to five hours at 60°C . to remove unchanged acetaldehyde, and the above tests were repeated. The reducing agent left was assumed to be formic acid.

Previous work on the oxidation of acetaldehyde has shown that under various conditions many different products can be obtained. Thus an aqueous solution of acetaldehyde was oxidized by means of cerium sulphate in the presence of dilute sulphuric acid to glycollic aldehyde and glyoxal, together with large quantities of formic acid and carbon dioxide (5). Oxidation with aqueous hydrogen peroxide gave a complex peroxide, which reacted further to give acetic acid and water (13). An acid solution of potassium permanganate yielded acetic acid alone, whereas an alkaline solution produced acetic acid, oxalic acid, and carbon dioxide, the relative proportions of the products depending on the alkalinity of the solution (8). Fewer products have been found as a result of the oxidation of gaseous acetaldehyde by means of air or oxygen, except in the presence of catalysts (2, 6, 12, 16, 17).

All investigators who have studied the oxidation of acetaldehyde by oxygen agree that the primary product is peracetic acid. They do not agree, however, on the mechanism by which it is formed, or the manner in which it subsequently reacts. Complete analyses of the products of the oxidation have never been reported.

The products found in the present investigation are as follows:—peroxygen compounds, formic and acetic acids, and carbon dioxide in small quantities

in the earlier stages of the reaction and in larger quantities together with carbon monoxide in the later stages. It is thus apparent that peracetic acid is not the sole product of the oxidation even in the earliest stages of the reaction. It is of interest to compare the quantities of the products formed at various stages of the reaction, in order to discover the significance of the pressure change. In Table I the products and pressure decreases are tabulated in terms of the percentage of the initial amount of acetaldehyde present.

TABLE I
THE PRODUCTS OF THE REACTION*
1 $\text{CH}_3\text{CHO} + 1.5 \text{O}_2$

Pressure decrease	Oxygen used	CO_2 formed	Condensable, including acetaldehyde	Total acids	Formic acid	Peroxygen compounds
1.6	20.2	5.9	113	—	—	—
10.0	29.8	6.6	113.4	20	15	6
22.1	40	7.1	111	—	—	—
30	—	—	—	50	17	12
48	62.3	6.4	108	—	—	—
50	—	—	—	60	13	8
70	—	—	—	80	0	11
90	—	—	—	90	0	11

*Temperature, 60°C .

It is apparent that up to a 50% pressure decrease constant amounts of carbon dioxide, formic acid, and peroxygen compounds are formed at all stages. The amount of oxygen used is remarkably large at the beginning of the reaction and finally increases in a manner corresponding to the increase in total acidity.

The products are not appreciably affected by a variation in temperature. Analytical results for 78°C . are given in Table II.

TABLE II
THE PRODUCTS OF THE REACTION AT 78°C .
1 $\text{CH}_3\text{CHO} + 1.5 \text{O}_2$

Pressure decrease	Oxygen used	CO_2 formed	Condensable, including unchanged acetaldehyde
3.2	25.0	5.5	116.0
19.7	38.0	8.8	109.3
44.4	68.5	6.7	117.5
46.4	70.0	3.9	119.9

Increasing the surface of the reaction vessel has a marked effect on the amount of carbon dioxide formed, and of oxygen used. As shown by Table III, a constant quantity of carbon dioxide is again formed at every stage of the reaction, and a constantly increasing quantity of oxygen is used as the reaction progresses.

TABLE III
ANALYSIS OF PRODUCTS FORMED IN PACKED BULB

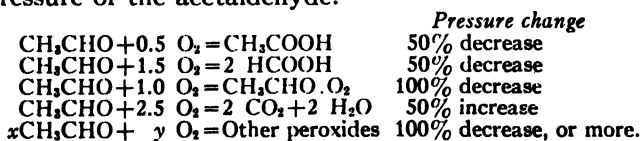
Temperature, °C.	Pressure decrease	Oxygen used	CO ₂ formed	Condensable, including unchanged acetaldehyde
60	0.20	26.0	9.0	117.5
60	0.75	42.0	18.0	123.0
60	23.2	55.5	14.0	118.0
60	46.5	67.5	15.6	105.7
60	76.0	84.5	11.7	97.5
78	0.56	34.0	19.0	115.0
78	50.0	84.2	24.2	110.0

It may therefore be concluded that at the beginning of the reaction, before the pressure has decreased to an appreciable extent, some carbon dioxide and formic acid are formed, the amount depending mainly on the surface of the reaction bulb, and to some extent on the temperature. These substances apparently do not continue to form during the later stages of the reaction, or at all events if they do form they break up again at an equal rate. The subsequent reaction, *i.e.*, the one which is accompanied by a pressure decrease, is an oxidation reaction since a constantly increasing amount of oxygen is used up during the pressure change.

The Pressure Change Accompanying the Reaction

As indicated before, the reaction is accompanied by a pressure decrease, the rate of which reaches a maximum only after a considerable time, especially at low temperatures. The maximum pressure decrease reached varies from 50 to 100% of the initial partial pressure of acetaldehyde, the percentage depending on the amount of oxygen present. At 60°C., however, the rate of pressure decrease does not reach zero but attains a constant low value after 100% decrease is reached, which is undoubtedly due to partial condensation of the products of the reaction. At higher temperatures after passing through a minimum the pressure begins to increase again. The present investigation, however, is concerned only with the first phase of the reaction, *i.e.*, the part which is accompanied by a decrease in pressure.

Oxidation of acetaldehyde according to the equations given below would result in the following pressure changes, expressed as percentages of the initial partial pressure of the acetaldehyde.



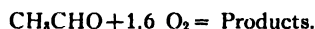
Since the pressure decrease always tends to a value of 100%, a relatively stable peroxygen compound must be the major product in the early stages of the oxidation. To decide what other reactions occur simultaneously other considerations must be taken into account.

Table IV shows the maximum pressure decreases reached under various conditions.

TABLE IV
MAXIMUM PRESSURE DECREASES

Temperature, °C.	$\frac{\text{O}_2}{\text{CH}_3\text{CHO}}$	Pressure decrease as per cent of partial acetaldehyde pressure	$\frac{\text{O}_2/\text{CH}_3\text{CHO}}{\% \text{ Pressure decrease}}$
80	1.20	82	0.0147
100	1.17-1.25	78	0.0155
100	1.34	88	0.0153
100	1.40-1.50	95	0.0153
100	1.50	100	0.0150
120	1.18-1.26	77	0.0158
120	1.32-1.36	81	0.0165
120	1.40-1.48	88	0.0164
120	1.53-1.57	98	0.0158
120	1.60-3.00	100	0.0160

It is thus apparent that if 1.6 times as much oxygen as acetaldehyde is used, a pressure decrease of 100% will always be obtained at 120°C., and this is also approximately true for 100° and 80°C. If a smaller amount of oxygen is used, the ratio $\frac{\text{oxygen/acetaldehyde}}{\% \text{ pressure decrease}}$ is constant for any given temperature. The maximum pressure decrease therefore depends almost entirely on the relative quantities of the reactants. The overall equation for the oxidation may therefore be written



The Mechanism of the Reaction

It has already been suggested by the authors and others that the major part of the reaction involves the combination of one molecule of acetaldehyde and one of oxygen to form a peroxygen compound. This would involve a pressure decrease of 100% as required, but it obviously expresses only one of several reactions which occur, since, as already shown, other products besides peroxygen compounds are formed, and more than one molecule of oxygen is used per molecule of acetaldehyde.

It is possible at this point to suggest what reactions occur (a) during the induction period, and (b) during the subsequent stages, on the basis of products found, oxygen used, and pressure change.

The following data were obtained in a typical experiment carried out in a reaction vessel packed with broken glass.

Temp., °C.	Initial acetaldehyde pressure, cm.	$\text{O}_2/\text{CH}_3\text{CHO}$	Pressure decrease	O_2 used	CO_2 formed	Condensable, including acetaldehyde
			Percentage of acetaldehyde partial pressure			
60	8.7	1.96	0.65	42	18.2	123.5

Assume that the following reactions occur simultaneously:

- (1) $\text{CH}_3\text{CHO} + 2.5 \text{ O}_2 = 2 \text{ CO}_2 + 2 \text{ H}_2\text{O}$,
 (2) $\text{CH}_3\text{CHO} + 1.5 \text{ O}_2 = 2 \text{ HCOOH}$.

To explain the products formed, these two reactions must occur to the following relative extents:

- (1) $9 \text{ CH}_3\text{CHO} + 22.5 \text{ O}_2 = 18 \text{ CO}_2 + 18 \text{ H}_2\text{O}$,
 (2) $13 \text{ CH}_3\text{CHO} + 20 \text{ O}_2 = 26 \text{ HCOOH}$.

The pressure change accompanying this is 2.5%, and the condensable products are 26 HCOOH, 78 CH₃CHO, and 18 H₂O, a total of 122%. No products other than formic acid and water could account for an amount of condensable products larger than 100% of the acetaldehyde used, and at the same time explain the very slight pressure change.

The above mechanism is confirmed by a large number of analytical results. The data for a typical experiment in an unpacked bulb at 60°C. are given below.

	Observed, %	Calculated, %		Observed, %	Calculated, %
Formic acid	15.0	(15.0)	Pressure decrease	1.6	2.2
Carbon dioxide	6.0	(6.0)	Condensable		
Oxygen used	20.2	18.7	products	112.7	110.5

We may therefore consider the mechanism of the processes occurring during the induction period to be established.

For the later stages of the reaction good agreement with experimental results is obtained if we assume that a mixture of the two peroxides, CH₃CHO.O₂ and (CH₃CHO)₂O₃, is formed. Table V gives a comparison of observed analyses with those calculated in this way. In every case the experimental values lie between those calculated for the two peroxides.

TABLE V
PRODUCTS OF THE LATER STAGES OF THE REACTION

Pressure decrease, %			Condensable products, %		
Observed	Calc. for AcO ₂	Calc. for Ac ₂ O ₃	Observed	Calc. for AcO ₂	Calc. for Ac ₂ O ₃
Temperature, 60° C.					
23.2	21	33	118	120	108
46.5	34	53	106	120	100
76.0	50	81	97	120	90
Temperature, 78° C.					
50.0	42	72	110	119	88

NOTE:—Packed reaction vessel. Partial acetaldehyde pressure, 13.5 cm. Oxygen/acetaldehyde ratio, 1.90.

Equally good agreement is obtained in other cases. It should, however, be pointed out that the suggested mechanism for this part of the reaction is by no means the only possible one.

The Velocity of the Reaction

The use of the pressure change as a measure of the rate of oxidation of acetaldehyde is justified by two facts. In the first place the amount of oxygen used increases regularly as the pressure decreases, as shown by Table II. In addition, the form of the pressure-time curves remains unaltered when the conditions under which the reaction occurs are altered. Fig. 2 shows that the curves obtained with different partial pressures of acetaldehyde and with different proportions of acetaldehyde and oxygen are affine. In a similar way Fig. 3 shows that the form of the curves is unaltered when the temperature is varied.

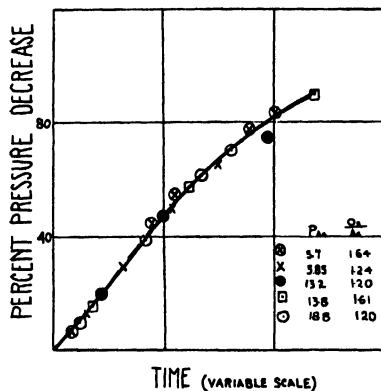


FIG. 2. Affine curves for 120°C., showing the effect of acetaldehyde pressure and of oxygen-acetaldehyde ratio.

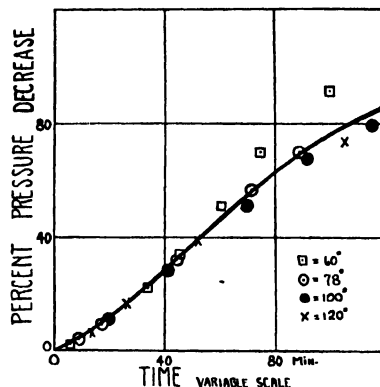


FIG. 3. Affine curves showing the effect of temperature. Partial acetaldehyde pressure = 9.0 cm. Oxygen-acetaldehyde ratio = 1.30.

Data for a typical run are given in Table VI.

TABLE VI
TYPICAL REACTION VELOCITY DATA
1 CH₃CHO + 1.62 O₂

Time, min.	Pressure decrease, cm.	Pressure decrease as per cent of initial acetaldehyde press.	Time, min.	Pressure decrease, cm.	Pressure decrease as per cent of initial acetaldehyde press.
0	0	0	23	11.27	77.1
3	1.45	9.9	28	12.47	85.4
6	3.25	22.3	32	13.15	90.1
12.5	7.10	48.6	44	14.45	98.8
17	9.23	63.2	51	14.65	100.2

NOTE:—Temperature, 120° C. Initial acetaldehyde pressure, 14.6 cm.

The effect of the total pressure on the rate of reaction is illustrated by

Fig. 4, in which pressure-time curves are given for the same mixture and temperature, but for different total pressures.

As stated before, there is an induction period which is particularly noticeable at low temperatures. In order to determine the effect of various factors on the rate of reaction it is customary to compare the time for some arbitrarily defined fractional change. In the present case the times for the reaction to proceed from 20 to 40% pressure decrease have been chosen in order to eliminate the effect of the induction period.

Table VII shows the effect of temperature, pressure, and the relative proportions of the reactants on the value of $T_{40} - T_{20}$. It will be seen from the table that the rate of reaction increases rapidly as the partial pressure of the acetaldehyde increases, but is independent of the partial pressure of oxygen. The order of the reaction with respect to acetal-

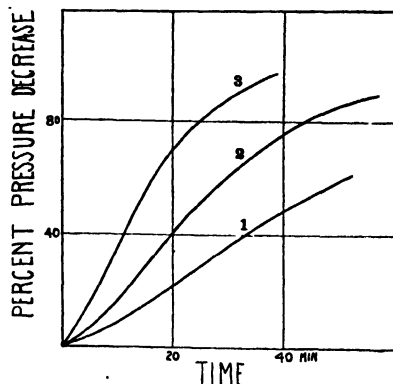


FIG. 4. Effect of total pressure on the rate of reaction. 120°C . $\text{O}_2/\text{Ac} = 1.60$
Curve No. 1 2 3
Acetaldehyde press., cm. 5.8 9.6 14.6

TABLE VII
THE EFFECT OF VARIOUS FACTORS ON THE RATE OF REACTION

Temp., $^{\circ}\text{C}$.	P_{ac} , cm.	O_2/Ac ratio	$T_{40} - T_{20}$, min.	Temp., $^{\circ}\text{C}$.	P_{ac} , cm.	O_2/Ac ratio	$T_{40} - T_{20}$, min.
120	18.3	1.21	6.0	120	9.6	1.95	8.0
120	14.7	1.18	6.5	120	5.8	1.64	14.0
120	12.8	1.20	7.5	100	14.8	1.17	18
120	5.5	1.26	11.0	100	11.5	1.25	19
120	15.1	1.34	7.0	100	8.4	1.18	27
120	10.1	1.32	7.0	78	18.8	1.18	24
120	15.2	1.43	6.5	78	13.3	1.20	30
120	9.3	1.48	7.0	78	8.2	1.23	47
120	5.4	1.42	14.0	60	18.9	1.25	29
120	15.2	1.57	7.0	60	10.9	1.28	76
120	9.2	1.53	9.0	60	5.75	1.30	110
120	14.6	1.62	5.0				

dehyde can be found in the usual way by plotting $\log P_{\text{Ac}}$ against $\log(T_{40} - T_{20})$ and measuring the slope of the line. The values thus obtained are given in Table VIII.

TABLE VIII
THE ORDER OF THE REACTION

Temperature, $^{\circ}\text{C}$.	120	100	78	60
Order	1.47	1.7	1.7	ca. 1.7

The Temperature Coefficient

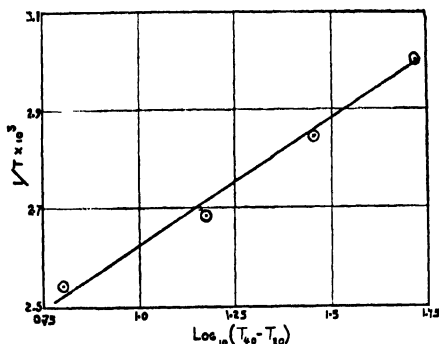


FIG. 5. The temperature coefficient of the reaction. $O_2/Ac=120$. Partial acetaldehyde pressure = 15.0 cm.

In Fig. 5 the reciprocal of the absolute temperature is plotted against $\log(T_{40} - T_{20})$. From the slope of the line the heat of activation is found to be approximately 8700 ± 700 calories. The data from which Fig. 5 was constructed are given in Table IX.

The Effect of the Surface of the Reaction Vessel

The effect of the surface was investigated by adding glass to the reaction vessel. In one series of experiments Pyrex tubing having an inner diameter of about 3 mm. was broken into lengths of 1-2 cm. and used. The total surface in this case was about five times the original surface. Two samples of crushed

TABLE IX
THE TEMPERATURE COEFFICIENT OF THE REACTION

Temperature, °K.	393	373	351	333
$T_{40} - T_{20}$, min.	6.5	15.1	27.9	51.9

Initial partial pressure of acetaldehyde, 15.0 cm.

glass were also used, both of which increased the surface to about ten times its original value. The effect of the increased surface on the reaction velocity is shown in Table X.

TABLE X
THE EFFECT OF SURFACE ON THE RATE

Temperature, °C.	Total surface Original surface	Sample of glass, no.	Rate Original rate
120	5	1	3.36 3.93 4.09
100	5	1	3.83 4.00
120	10	2	4.60 4.42
120	10	3	4.49 4.47 4.26

NOTE:— Acetaldehyde pressure, 13.5 cm. O_2/Ac ratio, 1.27.

It will be seen that an increase in surface increases the rate of reaction, but that a maximum effect is apparently reached when the surface is about five times that of the reaction vessel. A further increase in surface has little effect on the reaction velocity, but it does have some effect on the form of the pressure-time curves. This effect can be seen from the affine curves in Fig. 6, and consists mainly of an accentuation of the induction period.

An increase in surface also causes a change in the maximum pressure decrease accompanying the reaction, as shown by Table XI.

It is therefore apparent that the secondary reactions which cause the ultimate pressure increase are much more pronounced with the larger surface. The effect of the surface will be referred to again in the next section.

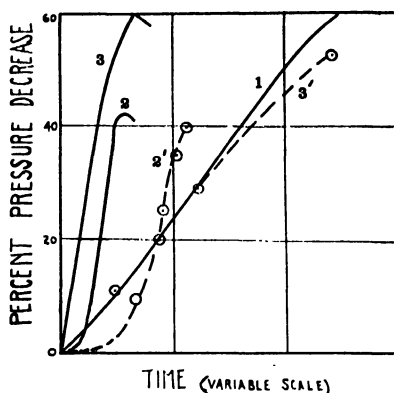


FIG. 6. *The effect of surface.*

Curve No.	1	2	2'
Total surface			
Original surface	1	10	10 (affine)
Curve No.		3	3'
Total surface			
Original surface		5	5 (affine)

TABLE XI
THE EFFECT OF SURFACE ON THE MAXIMUM PRESSURE DECREASE

Sample of glass, no.	Total surface Original surface	Maximum pressure decrease, %	Sample of glass, no.	Total surface Original surface	Maximum pressure decrease, %
1	5	61.5	2	10	45.9
3	10	48.9	none	1	77.0

NOTE:— Temperature, 120° C. Acetaldehyde pressure, 13.5 cm. Oxygen/acetaldehyde ratio, 1.28.

Discussion

In the first place, from the point of view of general theories of organic oxidation, it is apparent that the results obtained in this investigation are absolutely incompatible with any theory of the dehydrogenation type. The major step in the oxidation reaction is unquestionably the formation of a peroxide by the direct addition of oxygen. This is indicated both by the analytical results and by indirect considerations.

In virtually all gaseous oxidation reactions an induction period occurs. In order to explain the lag in reaching the maximum rate of pressure *increase*, it is customary to assume the intermediate formation of peroxides (7). In the present case an induction period also occurs; this is the reverse of the usual type, however, in that there is a delay in reaching the maximum rate of pressure *decrease*. Such an induction period obviously cannot be explained by the initial accumulation of peroxides. In a previous section it has been shown that the experimental data can be explained exactly on the assumption

that the reactions occurring during the induction period are not the same as those occurring in the later stages. While this is peculiar and no definite explanation can be given, it is possible to suggest how it may come about.

It has been shown above that an increase in surface tends to increase the rate of reaction and at the same time to accentuate the induction period. It seems probable therefore that the reactions occurring during the induction period are surface reactions. It will be shown later that the evidence points to the main reaction being of the chain type, with the chains initiated at the surface. The whole process will therefore be dependent on the condition of the surface. The adsorption of products from the reactions occurring during the induction period may therefore retard these processes and at the same time put the surface in a condition suitable for the development of the chain reaction. This suggested explanation is, of course, highly speculative. There is, however, no doubt that the reactions occurring during the induction period are quite distinct from, and cannot be the first step in, the subsequent reactions.

The kinetics of the oxidation of acetaldehyde have been previously discussed by Bodenstein (1). In Bodenstein's investigation a large excess of acetaldehyde was always used, so that the results are not entirely comparable with those reported here. The temperature coefficients of the two investigations, *viz.*, 10,000 and 8700 calories, are in as good agreement as could be expected. The general results are also in good agreement.

Bodenstein gives a complicated kinetic analysis of the reaction, but reports very little data to substantiate it, especially of an analytical kind. The main point in which the present investigation disagrees with Bodenstein's scheme is in connection with the induction period. This is explained in his paper as being due to the oxidation of impurities which act as inhibitors and are probably derived from stopcock grease. This assumption cannot possibly explain the present results. For example, in one case previously mentioned, at the end of the induction period 42% of oxygen had been used, and 18.2% of carbon dioxide formed. In addition, the total amount of condensable material had gone up to 123.5% of the initial quantity of acetaldehyde. These amounts of material cannot possibly have been derived from a trace of stopcock grease. The induction period was also found to be quite reproducible, which would certainly not be the case if it were due to the fortuitous presence of a trace of impurity.

Bodenstein's kinetic analysis of the oxidation of acetaldehyde will not be discussed further for several reasons. In the first place, it is rather complicated and has practically nothing to substantiate it. Secondly, there is no justification for dismissing the induction period as due merely to impurities. Finally, it has already been pointed out that the oxidation of acetaldehyde is attended by a number of complications. It is therefore a most unfortunate reaction to choose as a typical example on which to base a general theory of organic oxidations.

The oxidation of acetaldehyde is almost certainly a reaction of the chain type. It is true that an increase in surface increases the rate of the reaction,

but since the form of the pressure-time curves is altered, and especially since the effect of the surface reaches a maximum, the reaction is certainly not a simple heterogeneous one. The general trend of the results is analogous with those of other organic oxidation reactions of the chain type. Thus the order of the reaction is variable and not integral, and the rate is dependent on the aldehyde concentration and not on that of oxygen. The effect of increasing the surface indicates that the chains are initiated, at least largely, at the walls of the container. Since with increased surface a maximum rate is reached, the chains would also seem to be broken at the walls to a considerable extent.

The heat of activation of the reaction is much lower than that of a homogeneous bimolecular reaction occurring at the same rate at the same temperature. If, however, the primary step occurs at the walls of the vessel, this is not surprising since in the case of a heterogeneous reaction the heat of activation calculated from the temperature coefficient is only an apparent one, and will be lower than the true value by the heat of desorption of the reactants.

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THE VOLUME RELATIONS OF THE SYSTEM CELLULOSE AND WATER¹

BY EDGAR FILBY² AND O. MAASS³

Abstract

The absolute specific volume of cellulose has been determined by means of helium gas. A new experimental procedure has been developed, which, it is claimed, is more accurate than any previously used where a small quantity of porous material is involved. The method is extended to include the measurement of the volume of a system resulting from the adsorption of a vapor on a porous material, and applied in this instance to the system cellulose-water vapor. The specific volume of cellulose in helium was found to be 0.640 ± 0.001 cc. The volume of the system cellulose-water vapor was found to be much smaller than that given by the components cellulose and condensed water, as long as the amount of adsorbed water was below 4%. With more than 8% of adsorbed water the density of subsequently sorbed water was found to be the same as that of the normal liquid.

The hypothesis is put forward that the first water adsorbed on cellulose enters into a definite chemical combination, considerable contraction taking place, and that subsequently adsorbed water first causes an increase in cellulose surface, with further consequent contraction in the volume of the two components and then a filling up of the so-called capillary spaces in which the water maintains its liquid properties. The relation of this hypothesis to the hysteresis effect is indicated, the trend which further work should take is pointed out, and the interest of this problem from a practical point of view is discussed.

Introduction

The specific volume of cellulose has been under investigation since the time of Kopp (6) who used a "volumenometer" and air as a gaseous medium. The more recent and more trustworthy determinations of this property were made by the pycnometer method with various liquid media; Vignon (14), Guttman (5), de Mosenthal (10), Cross and Bevan (2), Little (9), Richter (11) and Lewis (8). A good average value for the specific volume of cellulose in water was found to be 0.621. In 1927, Davidson (3) investigated a number of cotton samples by means of a gaseous method in which helium was used. These measurements showed cotton cellulose to have an absolute specific volume of about 0.640, which is the helium value obtained in the present work where measurements were also carried out in air and hydrogen.

Urquhart (12, 13) emphasized the idea that in nature cellulose is precipitated in the presence of water, which probably results in a certain retention of water molecules by the hydroxyl groups of the cellulose crystallites. On drying, the residual valencies which were formerly occupied by water molecules tend to satisfy each other, and the cellulose micellae join together to form fibres, while on being hydrated, the water enters the spaces between these micellae, satisfying their secondary valencies, and thus causing a swelling similar to that suffered by gelatine and other swelling gels.

If, while wetting a cellulose sample, the water-vapor pressure be measured

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² Contribution from the Physical Chemical Laboratory, McGill University, Montreal. From a thesis presented by Edgar Filby in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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at different moisture contents, and then the same experiments be made while drying the sample, it is found that a hysteresis phenomenon takes place, that is, the relative vapor pressure on the desorption isotherm is found to be lower than that on the adsorption isotherm for the same percentage moisture. Urquhart explained this phenomenon by assuming that dry cellulose, on being exposed to water, satisfies its residual valencies, causing those valencies which mutually attract each other on drying to be released, and the gel to disperse. Thus on hydrating, the active groups increase, while on drying they decrease, in this way giving the pronounced hysteresis effect. He also assumed that "during hydration only a definite fraction of those residual valencies which satisfied each other on drying and distorted the crystallites will be released". Thus it would follow that the drier the cellulose, the less water it will take up on hydration, which seems to assign to cellulose some non-swelling properties. It will be noticed on any adsorption or desorption isotherm of cellulose and water, that the vapor pressure, which, in the initial stages of adsorption rises very slowly, shows a sharp increase at approximately 3 to 4% of water and maintains this until the saturation point is nearly reached. This and other properties show some difference in behavior on the part of the water initially added and that subsequently added to a so-called dry sample of cellulose.

Among the other properties referred to above, the sorption of hydrogen chloride on cellulosic materials containing sorbed water has given evidence that two states of sorbed water exist, namely, chemically combined surface water, and water which is held by capillary attraction between adjacent cellulose micellae. The cellulose micella presents a surface of active groups to which water adds in a chemical sense and thus loses its properties, except in so far as a dissociation equilibrium exists. For purposes of designation the authors propose to call this "chemically bound water"; water subsequently sorbed will be held under a reduced pressure by virtue of the proximity of adjacent cellulose surfaces, a pseudo-curvature for this liquid water being calculable on the basis of the Gibb's equation. Water in this second state can be expected to retain approximately the properties of the normal liquid, though there may be slight modifications since the surfaces of chemically combined water will in turn impose some modifications on the adjacent molecular layer of water, and since the "capillary conditions" involving internal pressure as caused by the curvature of the free liquid water are bound to have some influence. The vapor pressure of such water will of course be dependent entirely on the curvature of the free liquid surface. It is to be expected that marked changes in properties of the chemically adsorbed water will result. Among others, an apparent change in density will take place, while water subsequently sorbed, namely liquid water, will show no such change.

The present investigation will show that the above hypothesis has been verified. It may be convenient however, at this point, to indicate a further development which it is hoped to establish. The sorption isotherm for water on cellulose is quite different from the desorption isotherm, the latter taking place at lower relative humidities, indicating that on desorption, larger amounts

of water are held at corresponding relative humidities. In the opinion of the authors, the adsorption of water is accompanied by an increase in cellulose surface which may be supposed to take place as follows: the cellulose micellae are held together in the first place by the mutual surface attraction of cellulose for cellulose; the water first adsorbed satisfies the free cellulose surface and subsequently added liquid water is able to compete with the cellulose for cellulose attraction, thus increasing the effective surface. The experiments cited above with regard to hydrogen chloride adsorption indicate that this phenomenon actually does take place. The water entering between the cellulose surfaces becomes chemically bound and therefore, on desorption, is not subject to evaporation at the same vapor pressure as that at which it was adsorbed. It is suggested therefore that there is a larger amount of chemically bound water present in cellulose during desorption than during adsorption. In this way an explanation for the phenomenon of hysteresis is obtained, and it is hoped that density measurements on cellulose subjected to desorption will prove the above theoretical considerations.

It was the object of this investigation, therefore, to design and construct an apparatus which could be used for the examination of the volume relation of a two-component system resulting from the adsorption of an easily liquefiable vapor on an adsorbent material, and to carry out these measurements in the case of the system cellulose-water.

In order to accomplish this a number of experimental difficulties required solution. It was necessary that the measurements take place in an isolated medium which does not adsorb on the cellulose surface to any appreciable extent, that the vapor pressure to which the sample is subjected remain constant throughout the determination, and that the moisture content of the sample be measured under these conditions.

Apparatus

The novelty of the experimental procedure employed consists of making use of a gas-expansion method. In this method a gas is allowed to expand either into, or from, a cell containing the system under investigation, the pressure changes recorded being used for the purpose of density calculations. Previous methods employing a gas for the density determination of a condensed system had as their basis the injection of a known volume of gas into the space containing the system. This is neither as accurate as the expansion method, nor does it admit of detecting and correcting for adsorption of the calibrating gas. For convenience the description of this new method is arranged under the following sections: expansion apparatus, apparatus for moisture determination, pressure control, accessories, arrangement of apparatus, and general manipulation.

The Expansion Apparatus

A. Two cells of about 10 cc. capacity were connected together by an oblique stopcock. One cell was then connected to the vacuum line through a stopcock, while the other volume was attached to a differential mercury manometer which was in turn attached to a constant-level manometer. This

second cell was also connected through a stopcock, phosphorus pentoxide tube and calcium chloride tube to the atmosphere. This apparatus is shown at *A* in Fig. 2.

B. Another apparatus was constructed similar to *A* (Fig. 1—*B*) except that cell V_1 was calibrated with mercury and a side tube was attached to V_2 through which the cellulose could be introduced. This side tube was part of a 5-cc. graduated pipette and was calibrated with mercury before being attached to the apparatus.

C. In order to obtain easier access to V_2 , in which the cellulose was placed, a modified apparatus was built as shown in Fig. 1, *C*. In this the same general

arrangement was followed, one calibrated cell being attached to the vacuum line and to a second cell, which in turn was connected to the gas line and differential manometer. The main difference was that the cell V_2 was supplied with a finely ground lid which fitted on to a glass rim after the manner of a desiccator top. The lid was held down by a very thin layer of vaseline and sealed with a small mercury reservoir.

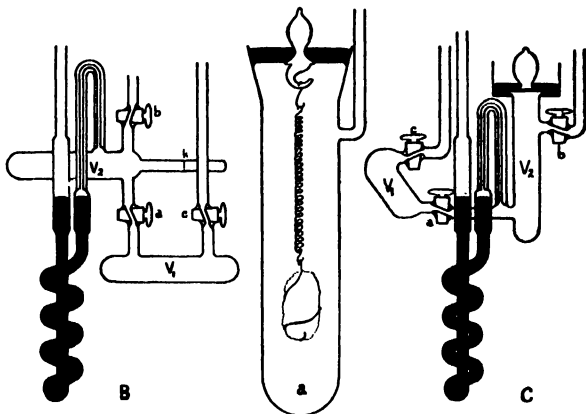


FIG. 1. Detailed expansion apparatus *B* and *C*, and spiral balance *a*.

The Apparatus for Moisture Determination

The moisture of the sample placed in V_2 was determined by measuring the elongation of a quartz spiral to which a sample of the same material was attached.

Two containers were made as shown in Fig. 1—*a*, and attached to the expansion apparatus as shown in Fig. 3. The containers were fitted with ground glass stoppers having a hook on the under side on which the quartz spiral could be hung. Measurements of the lengths of these spirals were made by means of a Becker cathetometer.

The quartz spirals were obtained by winding fibres on an automatic roller which operated like a screw, giving about 50 turns to the inch. The fibres were made from quartz rod which was heated in an oxy-acetylene flame and drawn out by attaching one end to a weight which was dropped about 12 ft. Fibres were made up to this length and found to be straight only when they were drawn out along a bench so that they were supported while cooling. The two spirals used in this work were tested for Hooke's law.

Many such spring balances have been set up and the sensitivity found to be proportional to the diameter of the helix and the number of turns per unit

length, and inversely proportional to the fourth power of the diameter of the fibre.

That is

$$S = K \frac{ND}{d^4},$$

where S is the sensitivity, N the number of turns per inch, D the diameter of the helix, d the diameter of the fibre and K some constant.

The Pressure Control

In order to keep the pressure equal on both sides of the differential manometer shown at A in Fig. 2, some device for introducing and extracting small

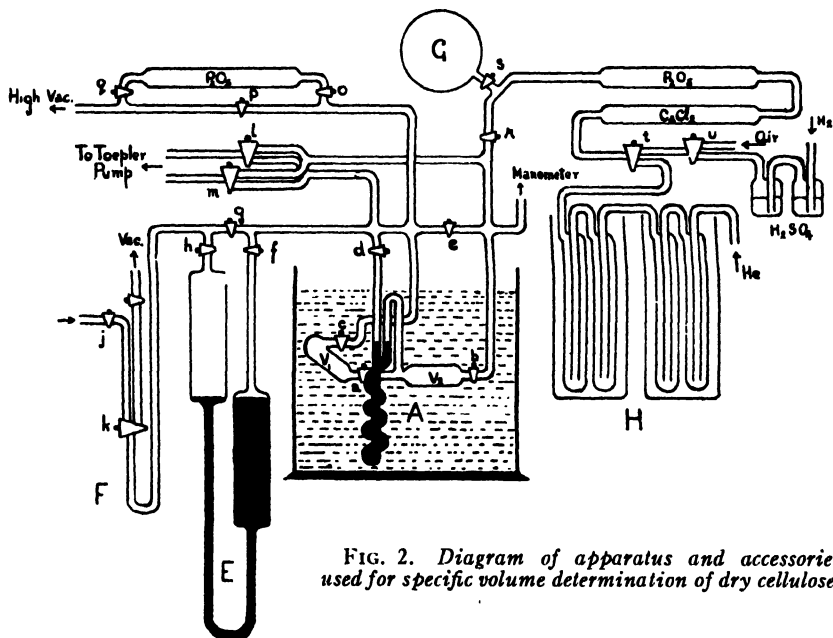


FIG. 2. Diagram of apparatus and accessories used for specific volume determination of dry cellulose.

quantities of gas from the left hand side of this manometer was needed. This was done through the arrangement of stopcocks shown at F in Fig. 2. As this apparatus is connected to the cylinder on the left hand side of U-tube E , which has a volume of about 800 cc., the introduction of a few cc. of gas at atmospheric pressure through stopcock k increases the pressure only very slightly. E is a U-tube both cylinders of which are of approximately the same volume, and was used to isolate the system from the pressure control when purified helium was being used as a gaseous medium.

Accessories

A Cenco Supravac consisting of a mercury-vapor pump backed by a Highvac oil pump was used in conjunction with an additional mercury-vapor pump. This combination gave a vacuum of less than 0.001 mm. of mercury in 10 min. when connected to a litre flask. A constant-level manometer was built and a thermometer to record temperature readings was attached to the mercury

column, behind which was placed a glass mirror scale. Low pressures were recorded on a McLeod gauge connected in series with this manometer. In order to purify helium four coconut-charcoal tubes, shown at *H* in Fig. 2, were made of Pyrex glass and hooked together in doublets so that they would fit into one-litre Dewar flasks. The tubes were filled with activated coconut-charcoal and connected to the apparatus and helium tank by de Khotinsky seals. To conserve the purified helium, a Toepler pump was built and attached to the line as shown in Fig. 2. Two automatic constant-temperature baths were set up to control the temperature of the expansion apparatus and the water bulb.

Arrangements of Apparatus

In Fig. 2 a diagrammatic sketch of the apparatus is shown. *A* is the thermostatically controlled expansion apparatus, the calibrated cell of which is connected through the stopcock *c* and the drying tube, to the Supervac pump. The cell *V*₂ has two outlets, one through the differential manometer and stopcock *d* to the constant-level manometer and pressure-control apparatus *E* and *F*, the other through the stopcock *b* to the manometer, which may be connected to the gas line through the stopcock *r*. The arrangement of stopcocks *l* and *m* which lead to the Toepler pump made it possible to pump gas to and from any part of the apparatus. The litre flask *G* was used for storing gas which was introduced into the apparatus through the drying tubes and the stopcocks *t* and *u*. A plug of cotton wool was used to filter out dust particles.

The apparatus for experiments using water vapor was set up as shown in Fig. 3, the water bulb being immersed in the usual constant-temperature water bath or Dewar flask containing an ice mixture, depending on the temperature required. This arrangement solved one of the main experimental difficulties, namely, the impregnation of the system to be examined for density with a definite known moisture content. The water vapor coming initially from *n* had to pass over the cotton on spiral *S*₁ then through the sample in cell *V*₂ and finally to the cotton on spiral *S*₂. When these two spirals *S*₁ and *S*₂ showed the same moisture content it indicated that the water-vapor pressure equilibrium had been established and that the cotton in cell *V*₂ had the same moisture content as that indicated by the two spirals. This point is emphasized to show the care taken to ensure that the moisture content of the cotton under investigation was definitely established.

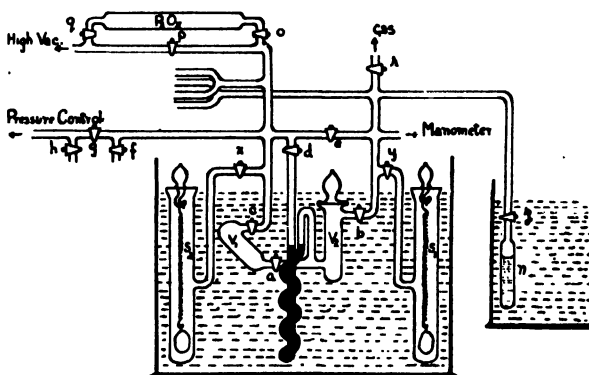


FIG. 3. Diagram of device added to specific volume apparatus for determination of amount of adsorbed water.

General Manipulation

Using expansion apparatus, *A*, and the set-up shown in Fig. 2, tests were made as to the reproducibility of results on expanding the gas in one cell at atmospheric pressure into the other cell when completely evacuated. This experiment was carried out as follows. The apparatus was opened to the atmosphere through the stopcocks *r*, *t* and *u*, thus obtaining the same pressure on both sides of the differential manometer, and the pressure was measured on the constant-level manometer at three different parts of the scale. A low-power microscope objective of about 60 cm. focal length was focused with the scale mark on the mercury meniscus at the right side of the differential manometer. Stopcocks *a*, *b*, *d*, and *r* were then closed and cell V_1 evacuated. When the pressure in V_1 as read on the McLeod gauge reached a low value of about .001 mm. Hg, stopcocks *c* and *o* were closed, *e* being left open. Next, the pressure on the manometer was adjusted to the pressure it was anticipated would be established in cells V_1 plus V_2 after the expansion had been made. When this was done, stopcock *d* was slowly opened, the mercury in the left side of the differential manometer gradually rising, until stopcock *a* was opened slightly, and the pressure on both sides of the manometer more nearly equalized. This operation took about one minute and great care had to be taken not to blow the mercury about. When stopcocks *a* and *d* were both open the pressure was adjusted through the control apparatus *F* until the mercury meniscus of the differential manometer was at the same scale division on the microscope as before, and thus the pressure on the constant-level manometer was the same as the pressure in cell V_2 .

When purified helium was used stopcock *g* was closed, thus isolating the system from the apparatus *F*, and causing the pressure to be controlled through the U-tube *E*.

In order to make experiments using water vapor as well as helium, water was placed in the bulb *n*, and freed from air by alternately freezing and evacuating several times. When the entire apparatus had been evacuated with stopcocks *r* and *z* closed, and the bath containing the water bulb had been adjusted to the required temperature, stopcocks *e* and *o* were closed, *a*, *b*, *c*, *d*, *x* and *y* being left open. Stopcock *z* was then opened, and the entire system left until the two samples attached to the quartz spirals had acquired the same percentage of moisture. When this water-vapor equilibrium was reached the vapor pressure was measured on the manometer, the stopcocks *a*, *b*, *c*, *x*, *y* and *z* were closed, *e* was opened, and the vacuum pump connected to the system to extract the water vapor from the surrounding glass tubing. The system was then isolated from the pump and drying tubes, and helium was introduced through *r* until a definite pressure was reached, when *r* was closed. The gas was allowed to flow into cell V_1 by opening *c* very slightly, thus avoiding a loss of water vapor from the apparatus. The pressure of gas in V_1 was measured as usual on the constant-level manometer, and the expansion carried out as before.

Materials

Mercury

Commercial mercury was purified by pouring it through a nitric-acid tower three or four times, and by washing with water. This mercury was then distilled in a Pyrex apparatus under a partial vacuum and in the presence of a slow stream of air. The mercury was redistilled into the manometer and Toepler pump in order to prevent any contamination from dust particles in the air.

Cellulose

A sample of purified rag cellulose, which had been soda-boiled was obtained from the Eastman Kodak Company, and was kept in a conditioning jar. Samples for moisture determination were taken whenever a sample was to be used in the expansion apparatus described above.

Preliminary Work

The apparatus shown at *A* in Fig. 2 was used to test the expansion method which was to be used in the actual experimental work. The experiment described under the heading "Manipulation" was repeated many times and concordant results obtained.

A number of experiments were carried out using an apparatus of type B (Fig. 1). These determinations gave a value of 0.63 and 0.61 cc. for the specific volume of cellulose in air and hydrogen respectively. In both cases the pressure in the cell containing the cellulose was initially about 1 atm., and was reduced to about $\frac{1}{2}$ atm. when the gas expansion was made.

Experimental Results

An apparatus of type *C* (Fig. 1) was found to be the most suitable for this work. With this, experiments were carried out in two ways: first, after the manner of those made in the preliminary work, where the pressure of gas on the cellulose was decreased from 1 to $\frac{1}{2}$ atm.; second, by having cell V_2 and the hole in stopcock *b* initially at vacuum pressure and cell V_1 at atmospheric pressure, then carrying out the gaseous expansion so that the pressure on the cellulose increased from about 0.001 mm. of mercury to about $\frac{1}{2}$ atm. The volume of cell V_1 was found before the apparatus was assembled, by filling with mercury and weighing at a given temperature (see Table I).

TABLE I
DETERMINATIONS OF THE VOLUME OF
THE CELL V_1

Wt. of Mercury, gm.	Temp., °C.	Volume of V_1 , cc.
137.133	28.0	10.1377
137.143	28.0	10.1384
137.143	28.0	10.1384
137.133	28.0	10.1377
Mean		10.1380

Calibration of V_2

After the apparatus had been assembled and attached in place of *A* in Fig. 2, 94.529 gm. of mercury was placed in the differential manometer and the volume of cell V_2 found by the expansion method previously described.

Letting p_1 be the initial pressure in cell V_1 in mm. of mercury, p_2 the initial pressure in cell V_2 in mm. of mercury, p_4 the final pressure in the combined volumes of V_1 and V_2 in mm. of mercury, T_1 the temperature of the constant-level manometer and P_1 , P_2 and P_4 be the pressures corresponding to p_1 , p_2 and p_4 after correcting to 0°C . for the expansion between mercury and glass, graphs being drawn for this from data taken from Landolt and Bornstein (7), then, if $P_1=0$, $V_2 = \frac{V_1 P_4}{P_2 - P_4}$ or, if $P_2=0$, $V_2 = \frac{V_1(P_1 - P_4)}{P_4}$ where V_1 and V_2 are the volumes of the respective cells in cc. Typical computations of V_2 are shown in Table II.

TABLE II
DETERMINATIONS OF THE VOLUME V_2

No.	p_2	p_4	T_1	P_1	P_4	V_2 , cc.
1	728.3	419.8	28.5	724.8	417.7	13.788
2	713.1	410.9	24.3	710.1	409.1	13.780
3	724.4	417.5	25.4	721.3	415.7	13.788
4	730.6	420.9	24.8	727.5	419.1	13.776
Mean						13.783

The lid of cell V_2 was taken off and replaced and the volume of the cell redetermined. The results of this determination are given in Table III and show that, as far as these experiments are concerned, the removal and replacement of the lid did not change the volume of cell V_2 .

TABLE III
DETERMINATIONS OF THE VOLUME OF V_2 AFTER REMOVAL AND REPLACEMENT OF THE LID

No.	p_2	p_4	T_1	P_2	P_4	V_2 , cc.
1	756.1	435.8	24.4	753.0	433.9	13.783
2	759.8	437.8	24.0	756.7	436.0	13.780
3	754.2	434.6	25.6	750.9	432.7	13.784
4	752.2	432.5	25.9	748.8	431.5	13.789
5	745.7	429.7	28.1	742.1	427.6	13.784
Mean						13.784

The cell V_2 was packed with a sample of moist cellulose, dry weight 4.805 gm., which was then dried for a period of three or four days by evacuating in contact with phosphorus pentoxide.

Experiments using Helium as a Gaseous Medium

For a few experiments, helium from the tank was purified by passing over activated charcoal at the temperature of liquid air. When the four charcoal tubes shown in Fig. 2 had been sealed into the vacuum line they were heated with a smoky flame and the adsorbed gases drawn off by the pump. After heating for three- or four-hour periods on several occasions a fairly high vacuum was obtained while the tubes were hot, this of course gave a high vacuum as soon as the tubes were cooled by immersing them in liquid air.

The pressure of helium was allowed to build up very slowly by adjusting the two stopcocks between the charcoal tubes and the helium tank. Two determinations made with purified helium are shown in Table IV. In these, the cellulose was initially evacuated and cell V_1 filled with purified helium at about atmospheric pressure. The data from experiments made with the dry unpurified gas are shown in Tables V and VI. In view of the close agreement between the results with purified helium and with impure helium (Table IV Nos. 1 and 2, Table V Nos. 1 and 2) it was considered quite unnecessary to carry out the purification, and in all further experiments the unpurified gas was used. In the first determinations of Tables IV, V and VI, the final pressure, P_4 , is recorded at various time intervals and the change in pressure after the initial expansion has taken place is given under the heading dp .

TABLE IV
RESULTS OBTAINED USING PURIFIED HELIUM AS A GASEOUS MEDIUM

No.	P_1	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	767.8	373.5 373.4 373.4 373.4	2 7 15 26	0.1	10.709	3.075	0.640
2	768.1	373.5	25	0.1	10.710	3.074	0.640

TABLE V
RESULTS OBTAINED WITH DRY UNPURIFIED HELIUM AS GASEOUS MEDIUM

No.	P_1	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	767.4	373.2 373.1 373.1 373.1	2 8 15 22	0.1	10.712	3.072	0.639
2	767.3	373.2	30	0.1	10.709	3.075	0.640
3	601.6	292.7	25	0.1	10.700	3.084	0.642
4	601.9	292.9	26	0.1	10.697	3.087	0.642
5	398.9	194.9	20	0.1	10.698	3.086	0.642
6	399.6	194.5	25	0.1	10.700	3.084	0.642

The above results show little variation of the specific volume of cellulose in helium over the pressure range investigated, also the almost instantaneous establishment of the equilibrium pressure. Both these facts indicate that helium is not adsorbed on cellulose, the small change of 0.1 mm. in the value

of dp being accounted for by diffusion of the gas into the inter-crystallite spaces.

TABLE VI
RESULTS OBTAINED USING DRY UNPURIFIED HELIUM AS GASEOUS MEDIUM

No.	P_1	P_4	Time, min.	dp	V_2	Volume of cellulose, cc. V	Specific volume
1	746.7	383.2 383.3 383.3 383.3	2 8 15 20	0.1	10.694	3.090	0.643
2	747.2	383.7	25	0.1	10.695	3.089	0.643
3	597.5	306.8	30	0.1	10.703	3.081	0.641
4	598.3	307.3	20	0.0	10.703	3.081	0.641
5	497.9	255.7	31	0.1	10.704	3.080	0.641
6	497.5	255.6	32	0.1	10.706	3.078	0.640

Experiments using Water Sorbed on Cellulose

Several determinations were made using helium as a gaseous medium and varying the amount of water sorbed on the sample. At first little or no success was met with in an attempt to obtain consistent results. Considering case No. 1 in Table VII for example; after the entire system had been evacuated and isolated from the pump and drying tubes, the water bulb was opened and the vapor allowed to pass over the suspended samples. When both samples recorded the same percentage of moisture, cell V_2 was isolated from the rest of the system and the water vapor removed from cell V_1 and the surrounding tubing by the vacuum pump and drying tubes. As can be seen from Table VII a gradual increase in pressure occurred, following the expansion, probably due to water vapor evaporating from the cellulose surface. This made it impossible to state accurately the percentage of water vapor held by the sample in cell V_2 .

The opposite effect is shown in case No. 2 in the same table. Here, after both suspended samples had acquired the same percentage of moisture, cells V_1 and V_2 were isolated and the water vapor allowed to remain in cell V_1 . The drop in pressure observed after expansion was evidently due to a sorption of water vapor by the cellulose in V_2 , since on filling V_1 with helium, some water vapor from the surrounding tubes would be swept into V_1 , giving a greater water vapor pressure there than in V_2 .

In order to avoid these sources of error, V_1 , as well as V_2 , was isolated from the system and water vapor withdrawn from the tubing through which the helium had to pass on its way to V_1 . This tubing was then filled with helium above atmospheric pressure, and the gas allowed to flow into V_1 very slowly so that little or no water vapor could escape from the cell. In this way

the vapor pressure in V_1 was kept the same as in V_2 as evidenced by the fact that scarcely any change of pressure was noticeable after the expansion of gas into V_2 had taken place. This method was followed in all subsequent work.

The data of Table VII show that the specific volume of the system cellulose and water is not additive in the initial stages of water sorption. The onus of this is thrown entirely on the water in order to simplify the representation of results. In Table VII, P_1 and P_4 represent the true pressure of helium in the apparatus, *i.e.*, the manometer pressure corrected to 0° C., minus the water vapor pressure in V_1 and V_2 , W_1 and W_2 represent the weight of water in grams sorbed per gram of cellulose on the quartz spirals S_1 and S_2 respectively, V_2 the calculated volume in cc. of the space around the cellulose in cell V_2 , V the total volume of sorbed water found by subtracting V_2 for the dry sample from V_2 for the wet sample, v the volume of this water in cc. per gram of cellulose, and d the average density of the sorbed water assuming the volume of the cellulose to remain constant under these varying conditions.

TABLE VII
AVERAGE APPARENT DENSITY OF ADSORBED WATER VAPOR AT VARIOUS
PERCENTAGES OF MOISTURE

No.	W_1	W_2	Vap. press.	P_1	P_4	Time, min.	V_2	V	v	d
1			4.6	761.1	374.0 374.4 374.9 375.4	3 17 40 80				
2			4.6	761.0	372.9 372.5 372.1 371.0	4 17 42 84				
3	0.0320	0.0318	4.6	755.5	368.6 368.6	4 25	10.644	0.058	0.0121	2.62
4	0.0325	0.0315	4.6	761.7	369.5 369.5	5 20	10.642	0.060	0.0125	2.56
5			0.0	753.9	370.3	20	10.502			
6			0.0	753.9	370.3	22	10.502			
7	0.0622	0.0618	10.5	726.3	358.9	5	10.377	0.125	0.0250	2.48
8	0.0634	0.0625	10.5	732.0	361.8	3	10.373	0.130	0.0260	2.42
9	0.1105	0.1074	15.4	725.9	363.0	3	10.140	0.362	0.0723	1.50
10	0.1590	0.1560	16.9	725.2	367.2	2	9.880	0.623	0.1240	1.27
11	0.1590	0.1570	16.9	725.2	367.3	4	9.879	0.624	0.1250	1.26

After experiment No. 4 in the above table the cell was repacked with cellulose because of an accident in which the mercury in the differential manometer was blown over into the cellulose. The mercury in the differential

manometer was not replaced by exactly the original weight and thus the volume of cell V_2 was slightly changed. However, this volume change is immaterial as the difference in volume of the space around the cellulose when wet and when dry is all that is required. The dry weight of this sample was 5.010 gm.

Discussion

The Specific Volume of Cellulose in Helium

The values given for the specific volume in purified helium (Table IV) show agreement with those in the unpurified gas (Table V), within the experimental error of about 0.005 cc. in the total volume of the sample, while the variation over a pressure range is sufficiently close to this error that no significance need be attached to it. It may be concluded that either the impurities in this sample of gas were too small to affect the volume of the cellulose to any measurable extent or that they were of a non-adsorbent nature. This is in agreement with the analysis of the gas which showed more than 95% of helium and less than 5% of hydrogen.

No effective adsorption of helium on the cellulose surface can take place as the specific volume remains practically constant over the pressure range, and the final pressure is almost instantly established. The value of 0.639 to 0.642 agrees very well with Davidson's (3) results of 0.638 to 0.642 for cotton boiled in soda and serves as a check upon the accuracy of the experiment.

The Density of Adsorbed Water Vapor

The data in Table VII which show the average density of the adsorbed water vapor at various percentages* of moisture, are plotted in Fig. 4 (top), and show an almost constant high density up to 4% of water, followed by a gradual drop along a line which appears to approach a density of one in an asymptotic manner at some high unattainable moisture content. Values may be obtained for the actual apparent density of water vapor being adsorbed at a particular percentage of moisture in the following manner. The volume of water adsorbed per gram of cellulose at a definite percentage of water vapor is subtracted from that adsorbed at another, so also are the corresponding weights of this water, and from these results the density of water being adsorbed at the mean value of the two percentages of moisture may be found. That is, if x be the total volume of water adsorbed on one gram of cellulose at $a\%$ of water and y be the volume corresponding to $b\%$, then the density of the water being adsorbed at $\frac{a+b}{2}\%$ of moisture is given by the equation

$$d = \frac{\frac{a}{100} - \frac{b}{100}}{x - y}.$$

This calculation was carried out at various intervals in Fig. 4 (top) and also for those recorded in Table VII. The figures are shown in Table VIII and are plotted in Fig. 4, (bottom).

*Here and subsequently "percentage" refers to cellulose by dry weight, that is, grams of water per 100 gm. of cellulose.

TABLE VIII
APPARENT DENSITY OF WATER VAPOR ADSORBED AT VARIOUS PERCENTAGES OF MOISTURE

From Table VI No.	Weight of water, $\frac{a}{100-b}/100$	Volume of water, $x-y$	% $\frac{a+b}{2}$	d $\frac{a-b}{100(x-y)}$
(3)	0.0319-0.0000	0.0121-0.0000	1.6	2.62
(4)	0.0320-0.0000	0.0125-0.0000	1.6	2.57
	0.030-0.020	0.0115-0.0076	2.5	2.56
	0.040-0.030	0.0155-0.0115	3.5	2.50
(8)-(4)	0.063-0.032	0.0260-0.0125	4.7	2.30
	0.060-0.050	0.0242-0.0194	5.5	2.08
	0.070-0.060	0.0300-0.0240	6.5	1.67
	0.080-0.070	0.0376-0.0300	7.8	1.32
(9)-(8)	0.109-0.063	0.0723-0.0260	8.6	0.99
	0.150-0.130	0.1155-0.0965	14.00	1.05
(11)-(8)	0.158-0.063	0.1250-0.0260	11.05	0.96

The curve obtained is continuous, with a sharp drop in density from a constant value of approximately 2.5 below 3% of adsorbed water to a value of 1.0 above 8%.

When the whole field of work on the relation between water vapor and cellulose is considered, one continually finds abrupt changes in behavior between 3% and 5% moisture content. For instance, the slopes of all adsorption and desorption isotherms change sharply between these two percentages, and the relation between cellulose and hydrogen chloride (4) shows that below 4%

of moisture, cotton adsorbs less hydrogen chloride than a dry sample under the same pressure, while above 4% of moisture the reverse is true.

In addition to the above, some conclusions drawn from crystal data and the density results may be of significance. If the average cellulose crystallite be considered to consist of 36 chains of 200 glucose units grouped together into a block 6 units by 6 units by 200, then there are 4872 units half exposed, which, upon the assumption of one water molecule for every completely exposed glucose unit, gives an adsorption of 2436 water molecules for 7200 glucose units, that is 3.7% by weight. Now the specific volume of cotton cellulose is

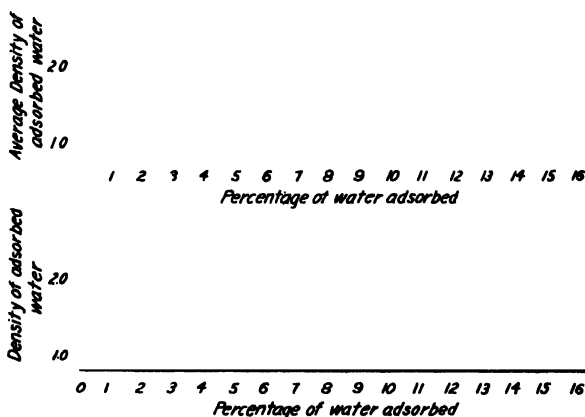


FIG. 4. Relations between apparent density of water and total amount adsorbed.

0.642 in helium and 0.621 in water*, a difference of 0.021 cc. in the volume of 1 gm. of cellulose in the two media. On a basis of this value of 3.7%, which is equivalent to 0.037 gm. of water per gram of cellulose, the volume of water which disappears per gram of water adsorbed, and takes up no space when cellulose is totally immersed in this liquid is $\frac{0.021}{0.037} = 0.57$ cc. From this it is

clear that 1 gm. of this kind of water occupies only $(1.00 - 0.57) = 0.43$ cc., that is, its density is about 2.4. Considering the assumptions made, the value agrees very well with that found experimentally in this work. The ordinate of the density curve begins to decrease at about 3 to 4% of moisture, but the density of the water does not decrease immediately to the normal value. This may be due to more than one water molecule being adsorbed per glucose unit, this second molecule being less firmly held than the first. However, it is more likely that the true size of the micellae is less than that used in the above calculation, large parts of their surfaces being held together by secondary valencies which are replaced by water, once the initially free surface has been saturated. This apparent density curve is therefore in full agreement qualitatively, and to a certain extent quantitatively, with the hypothesis of water sorption put forward in the introduction.

Water in the high-density phase adsorbs until the exposed surface is saturated, *i.e.*, until about 3 to 4% of moisture has been adsorbed. Then, further water sorbs in the normal phase until, by its swelling action, it splits apart the micellae, creating new surfaces which can adsorb more water in the high density phase. If this supposition be true, the action would continue until the entire surface of all the micellae was exposed to water vapor, and from then on, the water would adsorb in the normal phase of density one, which takes place when about 8% has been adsorbed. According to this theory of water adsorption, a hysteresis effect should be noticeable in the density relation shown in Fig. 4, that is, if the density were determined at successive moisture contents as the cellulose was desorbing water, then the density corresponding to the moisture content between the extremities of the curve (3% and 8%) should be greater than that represented in Fig. 4, where the determinations were made as the cellulose was sorbing water.

It is very probable that the whole process of paper making is dependent on forces that come into play between cellulose fibres, the entanglement theory being relegated to second place in comparison with the theory of secondary valencies (compare Urquhart (12,13) Campbell (1)). Since the removal of water plays an important part in bringing into action these secondary valencies, any information concerning the binding force between cellulose and water is of considerable interest. Measurements are to be made on the heats of adsorption of water on cellulose at various concentrations, which, when combined with the density-volume changes investigated in this work, will make possible an estimation of the magnitude of this binding force.

*The cellulose is completely immersed in water as is the case when the pycnometer method of specific volume determination is used.

In conclusion, it may be well to point out that the first addition of water to cellulose may be looked upon as a chemical combination, the volume of the water-cellulose compound formed being less than the sum of the volumes of the reactants, showing a definite contraction to have taken place. For convenience this effect has been tabulated in terms of the density of the adsorbed water.

Acknowledgment

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THE FREEZING OF WATER IN FISH MUSCLE AND IN GELATIN¹

By J. H. MENNIE²

Abstract

From the calorimetric measurements of Chipman and Langstroth (4) an estimate is made of the percentage of water frozen at temperatures down to -20° C. in samples of muscle from different species of fish. It is pointed out that if such measurements are to be used for the estimation of "bound" water, it must be known what portion of the water is kept from freezing by any inorganic salts that are present. Heat-capacity measurements on a 1.7% sodium chloride solution are given, and are shown to agree well with values calculated from available thermal data with the aid of the freezing-point curve. Heat-capacity curves for dry gelatin and 20, 40 and 66% gels are given. The unfrozen water in these gels at temperatures down to -20° C. is estimated and compared with results obtained by other methods.

Unfrozen Water in Fish Muscle

When animal or vegetable materials are preserved by freezing, only a part of the water which they contain is actually converted into ice. This is due partly to the lowering of the freezing point of the juice by the inorganic salts which it contains and partly, no doubt, to the "binding" of water by colloidal proteins. The question as to what proportion of the total water present is frozen at any given temperature is of interest in connection with the practical problems of freezing and cold storage.

Kallert (7) quotes some calorimetric measurements by Plank (12) from which he calculates that in meat 80% of the water is converted into ice at -10° C., 91% at -20° C., 99.9% at -50° C. and 100% only at -55° C. The calorimetric method was apparently used first by Müller-Thurgau (10, 11)† who made some measurements on apples and potatoes. Rubner (16)‡ and Thoenes (19) applied it to *Laminaria* and to various animal materials. Thoenes also made some measurements on gelatin and agar gels. The procedure described by Thoenes has been followed with slight modifications by Robinson (13, 14, 15) in a study of winter hardiness in certain classes of insects, and by St. John (18) in determining the unfrozen water in samples of egg white.

The total heat required to bring a sample of the material from some initial temperature T_1 below the freezing point to a final temperature T_2 above the freezing point, is measured in a calorimeter. From a knowledge of the specific heat of the material, the heat required simply to warm it through the temperature interval $T_2 - T_1$ is found. The difference between this and the observed value is attributed to the latent heat of fusion of the ice present, whence the weight of ice is obtained. Subtracting this from the total water content of the material gives the amount of water which remains unfrozen at the temperature T_1 .

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†Quoted by Jones and Gortner (6). ‡Quoted by Thoenes (19).

The equation given by Thoenes (19) and used with slight modifications by Robinson (13, 14, 15) and St. John (18) may be written

$$x = \frac{H - MS(T_2 - T_1)}{L_0 + T_1(C_w - C_i)},$$

where x is the weight of water frozen at the temperature T_1 (T_1 being given its correct algebraic sign) in a sample of material of weight M . H is the heat absorbed as measured in the calorimeter, S is the specific heat of the material, L_0 is the latent heat of fusion of ice at 0°C . and C_w and C_i are the average specific heats of water and ice respectively between 0° and T_1 .

This equation is discussed in detail in another paper (5) where the values 79.40 for L_0 and 0.55 for $(C_w - C_i)$ are recommended instead of 80 and 0.5, respectively, used by Thoenes. With the adoption of these constants, the equation has been used to calculate, from the measurements of Chipman and Langstroth (4), the percentage of water frozen in samples of muscle from different species of fish. The results are shown in Table I, in which the heat capacities given are average values read from the smooth curves. The equation is at best an approximation; the extrapolations on which it depends can be valid only for a comparatively short distance below the freezing point. Consequently, although Chipman and Langstroth carried their heat capacity measurements down to -70°C ., no calculations have been made for temperatures below -20°C .

In the last column of Table I the amount of unfrozen water is shown, expressed in grams per gram of total solids. The samples of skate, herring and mackerel contained an appreciable quantity of fat (1.8, 13.3 and 25.2% res-

TABLE I
FROZEN AND UNFROZEN WATER IN FISH MUSCLE FROM HEAT CAPACITY
MEASUREMENTS OF CHIPMAN AND LANGSTROTH (4)

Temp., °C.	Heat capacity, calories	Water frozen, %	Water unfrozen, gm.	Heat capacity, calories	Water frozen, %	Water unfrozen, gm.
<i>Cod (80% water)</i>				<i>Haddock (80% water)</i>		
-5	72.30	81.4	0.75	72.3	81.4	0.75
-10	80.75	91.3	0.35	79.8	89.6	0.41
-20	88.30	96.3	0.15	86.5	93.0	0.28
<i>Burbot (83% water)</i>				<i>Skate (80% water)</i>		
-5	75.6	83.6	0.80	60.0	61.4	1.70
-10	82.4	90.6	0.46	74.7	81.0	0.84
-20	98.8	93.7	0.30	84.2	88.8	0.50
<i>Herring (65% water)</i>				<i>Mackerel (58% water)</i>		
-5	67.5	90.5	0.29	54.4	78.6	0.72
-10	74.0	98.2	0.06	64.3	95.2	0.16
-20	80.0	99.9	0.00			

pectively) and a correspondingly lower proportion of water. In these cases the amount of fat was subtracted from the total solids and the figures given show the relation of unfrozen water to solids other than fat. The figures for cod, haddock and burbot are noticeably lower than those obtained for a 19.5% gelatin gel (Table V). Apparently there is less bound water in fish muscle than in a gelatin gel of similar water content. However, it must be added that the unfrozen water is a small difference between frozen and total water. Chipman and Langstroth give average values for the total water content and a small variation in it would mean a relatively large alteration in the figure for unfrozen water.

Most of the previous calorimetric measurements already mentioned were made with the object of estimating the amount of water bound by the colloidal material used. The unfrozen portion of the water has generally been assumed to be bound. In animal or vegetable tissues more or less inorganic matter is always present; fish muscle, for instance, contains 1 to 2% of salts, largely sodium chloride. In any salt solution the water will not all freeze until the temperature of the eutectic point is reached. Hence in a system which contains inorganic salts, at temperatures above the eutectic the unfrozen portion of the water will include the amount necessary to hold in solution at that temperature the salts which are present. The eutectic temperature of sodium chloride is -21.1°C . At a temperature even a fraction of a degree above this, the weight of water prevented from freezing by the salt is over three times the weight of the salt itself. The eutectic temperature of muscle juice is not known but is probably not above -20°C . (17). As most of the measurements made by the calorimetric method have been at temperatures not lower than -20°C ., the effect of even small amounts of inorganic salts cannot be safely ignored.

Heat Measurements on Salt Solutions

Barnes and Maass (1) have made some heat capacity measurements on potassium chloride solutions and have shown that the heat capacity could be calculated with considerable accuracy from existing data on the specific heats of the components and the heat of solution. Their calculation was made for a solution of nearly the eutectic composition and at a temperature below the eutectic. It was thought worth while to make some measurements on a dilute salt solution and to compare the observed values with a heat capacity curve derived from available thermal data with the aid of the freezing-point curve.

A solution containing 1.7% of sodium chloride was used. The heat capacity measurements were made in an adiabatic calorimeter which had been constructed by Dr. H. Ritchie Chipman and used by Chipman and Langstroth (4). The calorimeter itself and the method of operation are described in their paper. The solution was placed in a cylindrical brass container which was closed by a screw cap. This was suspended by a thread and lowered into a brass cylinder, closed at one end, into which it fitted closely. The cylinder was immersed in the cooling bath which consisted of ice and brine for temperatures down to -10°C ., and ether cooled with solid carbon dioxide for lower temperatures.

The bath was hand-regulated by the addition of ice or of carbon dioxide as required, and the temperature was read on a toluol thermometer. At least an hour was allowed for the solution to come to the temperature of the bath, then the container was drawn out by the thread and transferred as quickly as possible to the calorimeter. From the known heat capacity and the fall in temperature of the calorimeter the heat absorbed by container and contents was found. This was corrected in all cases to the same final temperature. The heat capacity of the container alone for the same temperature interval, found in exactly the same manner, was subtracted. Dividing by the weight of solution in the container then gave the heat capacity of the solution per gram. Table II shows the values obtained for the heat capacity per gram between various initial temperatures and a final temperature of 20° C. The weight of solution used was 10.892 gm.

TABLE II
HEAT CAPACITY PER GRAM OF A 1.7% SODIUM CHLORIDE SOLUTION BETWEEN
VARIOUS INITIAL TEMPERATURES AND A FINAL TEMPERATURE OF 20° C.

Initial temp., °C.	Heat capacity, calories		Initial temp., °C.	Heat capacity, calories	
	Obs.	Calc.		Obs.	Calc.
0.0	19.1	19.5	-22.2	107.9	108.7
-1.5	54.2	50.0	-22.5	104.6*	104.8*
-1.5	61.5	50.0	-22.7	104.9*	104.9*
-2.0	63.0	61.5	-23.7	105.1*	105.6*
-5.7	86.4	88.1	-24.4	105.4*	106.0*
-10.5	95.1	95.3	-24.5	107.9	109.8
-15.4	99.9	99.9	-24.7	108.7	109.9
-17.3	101.4	101.4	-41.3	116.6	117.2
-19.7	103.1	103.0	-65.4	126.1	126.8
-21.5	107.7	108.3			

*Supercooled.

The heat capacity was calculated in the manner suggested by Barnes and Maass (1). For temperatures below the eutectic the equation they give was used. This may be written:

$$H = aC_k(T_2 - T_1) + (1 - a)(C_w T_2 - C_i T_1 + L_o) + aS,$$

where T_1 and T_2 are the initial and final temperatures, a the weight of salt per gram of solution, C_k the specific heat of the solid salt, C_i the average specific heat of ice between T_1 and 0° C., C_w the average specific heat of water between 0° C. and T_2 , L_o the latent heat of fusion at 0° C. and S the heat of solution per gram of salt at T_2 . The values for C_w , C_i and L_o were taken from the paper of Barnes and Maass (2) and those for C_k and S from the International Critical Tables.

When T_1 lies above the eutectic, the water is not all frozen and a slightly different equation must be used. The warming of the solution from T_1 to T_2 may be regarded as proceeding by way of the following steps: (a) The concentrated solution (salt plus unfrozen water) at T_1 is warmed to T_2 . (b) The ice is warmed from T_1 to 0° C., melts at 0° C., and the resulting water is warmed

from 0°C. to T_2 . (c) The concentrated solution is diluted at T_2 by the water from the melted ice. The total heat required is then given by:

$$H = (a+x)C_s(T_2-T_1) + (1-a-x)(C_wT_2 - C_iT_1 + L_0) + D.$$

Here x is the weight of water remaining unfrozen at T_1 , which may be found from the freezing-point curve, and C_s is the specific heat of a salt solution

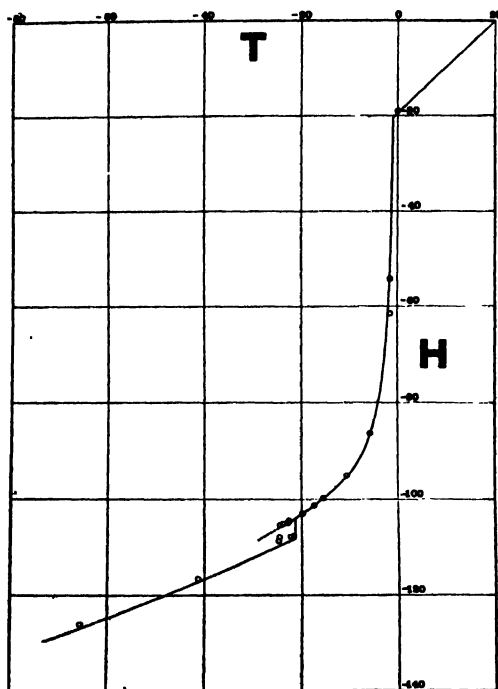


FIG. 1. Heat capacity curve for 1.7% sodium chloride solution.

containing a gm. of salt in x gm. of water. D is the heat of dilution at T_2 when enough water is added to $(a+x)$ gm. of this salt solution to make the total weight 1 gm. The freezing-point data and the values of C_s and D were taken from the International Critical Tables. The heat of dilution in no case exceeds 0.2 cal. and has been neglected in making the calculations.

From these equations a number of points were calculated and plotted, giving a smooth curve. The figures given as "calculated" in Table II were read from this curve. The calculated curve and the experimental points are shown in Fig. 1, which shows very clearly the excellent agreement between the observed and calculated results. Its most interesting feature is the evidence it exhibits of supercooling of the liquid or perhaps more cor-

rectly, supersaturation, below the eutectic point.

Briggs (3) has recently drawn attention to the necessity of considering the presence of inorganic salts in calculations of bound water. He gives the results of some calorimetric measurements on sodium chloride solutions from which he calculates the unfrozen water by means of Thoenes' equation. One of his solutions contained 1.7% of salt. For the sake of comparison his method

TABLE III
UNFROZEN WATER PER GRAM OF SALT IN A 1.7% SODIUM CHLORIDE SOLUTION

Author			Briggs		
Temp., $^{\circ}\text{C.}$	Calc.	Obs.	Temp., $^{\circ}\text{C.}$	Calc.	Obs.
-15.4	4.22	4.06	-15	4.50	4.99
-19.7	3.46	3.16	-20	3.46	3.24

of calculation has been applied to the figures at -15° and -20° C. taken from Table II. The result is shown in Table III. The "calculated" values were derived from the freezing point curve and the "observed" values from the heat-capacity measurements. The figures which Briggs actually gives apply to 1 gm. of solution and have been recalculated for 1 gm. of salt.

Heat Measurements on Gelatin

A number of measurements were made on gelatin gels of various concentrations. The gelatin used was Difco "Bacto-gelatin". By drying to constant weight at 110° C., it was found to have a moisture content of 14.7%; the ash content was 2.8%. Heat capacity curves were obtained for 19.5, 40.5 and 66.3% gels, and also for the dried gelatin.

The 19.5% gel was made up by weight, allowance being made for the water content of the gelatin, and was warmed on the water-bath until of uniform consistency. The liquid was then poured into a glass tube of slightly smaller internal diameter than the brass container. The tube was sealed and heated for 30 min. at 110° C., to sterilize the contents. When the gel had set, it was frozen quickly in carbon dioxide and ether. On freezing, the expansion of the gel burst the glass tube, whereupon the glass was removed and the solid cylinder of frozen gel dropped into the container and the cap screwed on. This procedure was adopted to avoid bursting the brass container on freezing the gel. The more concentrated gels did not expand so markedly and were made up directly in the container. A weighed amount of gelatin was placed in the container and the calculated amount of water added. The lid was then screwed on and the whole heated in the oven at 110° C. until the gel was of uniform consistency. In order to determine the period of heating necessary, similar amounts of gelatin and water were sealed in a glass tube and placed in the oven with the container. When the gel in the glass tube appeared to be uniform it was assumed that the same condition existed in the container.

The first sample of 19.5% gel was left in a cold-storage room at about -19° C. between runs. With the succeeding samples (except the dry gelatin) supercooling was avoided by freezing the gel first in carbon dioxide and ether and then allowing it to warm up to the initial temperature in the constant-temperature bath. From this point the procedure was the same as already described. The results are shown in Table IV and in Fig. 2.

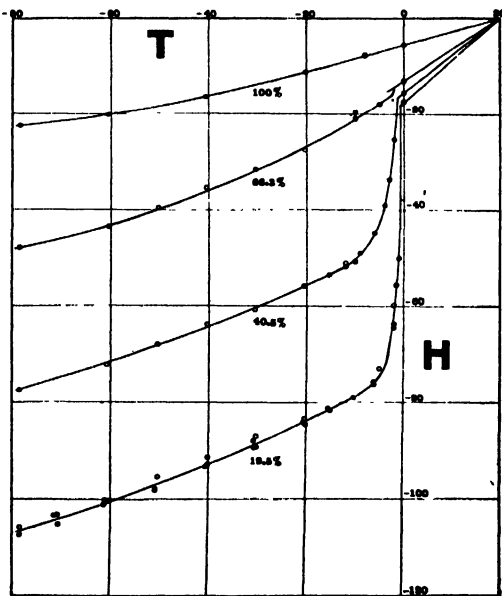


FIG. 2. Heat capacity curve for gelatin gels.

Two or three runs (indicated by an asterisk in Table IV) were made at temperatures a few degrees below zero with the container cooled directly to the initial temperature in the constant-temperature bath without the preliminary freezing mentioned above. In each case the points lie on, or very close to, the extension of the straight line drawn through the 0° C. point, showing supercooling to have occurred. To avoid confusion, only one of these points, *viz.*, that for the 66.3% gel, has been shown in Fig. 2. For the same reason, the point at -2° C. for the 66.3% gel is also omitted.

TABLE IV
HEAT CAPACITIES OF GELATIN GELS FROM VARIOUS INITIAL TEMPERATURES
TO A FINAL TEMPERATURE OF 20° C.

Initial temp., °C.	Dry gelatin	66.3% Gel	40.5% Gel	19.5% Gel (1)	19.5% Gel (2)	Initial temp., °C.	Dry gelatin	66.3% Gel	40.5% Gel	19.5% Gel (1)	19.5% Gel (2)
Heat capacity						Heat capacity					
0.0	5.6	13.0	15.5	17.6		-20.4			55.9		
-1.0					50.2	-20.5				84.1	
-1.5					55.7	-30.2					89.2
-2.0		16.5	25.3	20.2*	59.8	-30.3					87.0
-2.0				63.7		-30.5		31.7			
-2.0				64.5		-30.6					
-3.0			33.7			-30.7				87.9	
-4.0			39.0			-40.2				89.4	
-5.0		17.9			72.9	-40.2					91.3
-6.0			20.4*	75.6		-40.4			63.8		92.8
-6.0			44.8	76.1		-40.5		35.4			
-8.0	7.7					-40.6	16.4				
-8.0	7.9					-40.7				93.2	
-9.0			49.0			-50.2		39.7			
-10.0		19.6*	50.7		77.2	-50.4			68.0		96.3
-10.0		20.9	50.8			-50.8				97.8	
-10.0		21.1	50.9			-50.9				98.2	
-10.2				79.0		-60.4		43.5			
-12.0			51.1			-60.6	20.2				100.3
-12.0			51.8			-60.8			72.2		
-15.1					81.7	-61.1				100.2	
-15.5			53.6	81.3		-61.3				101.1	
-20.2					84.5	-70.9					103.2
-20.3	11.2	27.6			83.4	-71.5				103.4	
						-78.5	22.4	47.9	77.5	107.4	105.9

*Supercooled.

Two samples of 19.5% gel were used, and in Fig. 2 the points obtained with the first are indicated by black circles and with the second by plain circles. Both were prepared at the same time, but one was stored for a month in a sealed glass tube before being frozen and inserted in the container. There is no evidence that this prolonged storage influenced the amount of water frozen, since both sets of measurements fall on the same curve. Repeated measurements at the same temperature showed satisfactory agreement, with no indication of an alteration in the amount of water frozen as a result of repeated freezing and thawing. When removed from the container the gels appeared clear and uniform and there was no sign of any separation of water. These

facts are in agreement with the observations of Jones and Gortner (6) made on the same material (Difco "Bacto-gelatin") by the dilatometer method. They found that repeated freezing and thawing or storage up to five days at room temperature did not alter the amount of water which would freeze.

They concluded from their results that all the water which could be frozen down to -50°C. was frozen at the initial freezing temperature (about -6°C.), if sufficient time was allowed for establishment of equilibrium. That is, when equilibrium was once established at about -6°C. , no more ice was formed when the temperature was lowered further. On the other hand, Moran (8, 9) found by separating the ice which forms entirely on the exterior of a thin disk of gel when it is slowly frozen, that the unfrozen portion contained 54.3% of gelatin at -3°C. and the concentration increased to 65.5% (corresponding to 0.53 gm. water per gm. gelatin) at -19°C. , after which further lowering of the temperature produced no further changes.

TABLE V
UNFROZEN WATER IN GELATIN GELS

Temp., $^{\circ}\text{C.}$	19.5% Gel		40.5% Gel		66.3% Gel	
	Heat capacity, cal.	Unfrozen water, gm.	Heat capacity, cal.	Unfrozen water, gm.	Heat capacity, cal.	Unfrozen water, gm.
-3	69.0	0.92	34.0	0.96	16.4	0.48
-5	74.6	0.62	42.4	0.73	17.9	0.48
-10	78.8	0.51	50.4	0.57	21.0	0.48
-15	81.5	0.50	53.3	0.56	24.0	0.48
-20	83.8	0.50	55.6	0.58	26.9	0.49

In Table V is shown an estimate, made by means of Thoenes' equation in the form already used, of the water which remains unfrozen at temperatures down to -20°C. The heat capacities shown in Table V are taken from the curves of Fig. 2. The unfrozen water is expressed as grams per gram of dry gelatin. There is unmistakably a gradual freezing out of water down to some temperature between -10° and -15°C. There can be no question here of failure to establish equilibrium, since the gels were immersed in carbon dioxide and ether for at least half an hour before being brought to the initial temperature for each run. The amount of water remaining unfrozen at -20°C. is distinctly less than that found by Jones and Gortner (6), namely: 0.70, 1.05, 1.90 and 4.675 gm. in 32, 16, 8 and 2% gels respectively. The results in Table V are in quite good agreement with Moran's work (8).

On the basis of Moran's results (8) it was expected that the 66.3% gel might not freeze at all. Some of the water in it did freeze, however, as the break in the curve (Fig. 2) shows. Apparently this freezing all took place a little below 0°C. and further lowering of the temperature seems to have caused no increase in the amount frozen. It is possible that the somewhat prolonged heating used in the preparation of this gel resulted in some hydrolysis of the gelatin.

The general appearance of the curves in Fig. 2 is in agreement with Moran's (9) conclusion that there is no further freezing out of water at temperatures below -20°C . Calculations made with Thoenes' equation from the measurements at the lower temperatures are useless and other methods of calculation require a knowledge of the specific heats of the dry gelatin and of the unfrozen water (5). It was expected that the latter could be derived from further measurements on a gel of 66% or higher concentration, but the work was interrupted before this had been done. The measurements are now being repeated with improved equipment and technique, and using ash-free gelatin.

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HEAT CAPACITY MEASUREMENTS ON GELATIN GELS¹. I.

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Abstract

Heat capacity measurements using an adiabatic calorimeter have been made on a 24% gel, on dry gelatin and on gelatin containing 12.5% water. The calculation of bound water from calorimetric measurements is discussed, and the limitations of the method used by previous workers indicated. A new equation is derived and an estimate of bound water in the 24% gel given. Further work is in progress.

Introduction

Although there is ample evidence for the existence in lyophilic colloid systems of what is called "bound" water, very little is definitely known concerning the nature and intensity of the forces by which the water is held. In fact, there is no really adequate definition of bound water. When, for instance, a gel is brought to a low temperature, only part of the water present is found to freeze. The unfrozen portion is generally assumed to be "bound" by the colloid. Various other methods of estimating bound water are similarly based on the assumption that the bound water is that portion of the water content of the system which fails to display the normal behavior of ordinary "free" water under the particular experimental conditions used. The result is, according to Briggs (5), that "there are almost as many definitions of bound water as there are methods for determining it."

In a comprehensive review of the problem of water-binding in colloids, Kuhn (9) enumerates various ways in which water might be pictured as held by the colloid. These are not necessarily all distinguishable one from another but represent rather binding forces of varying intensity. He regards all the water contained in a gel as "bound" by the colloid and considers that the various physico-chemical methods which have been employed for the determination of bound water give only an estimate of the intensity of the binding forces.

This is essentially the same as the view advanced by Briggs (5) on thermodynamic grounds. He considers that the amount of water associated with or bound by a given quantity of colloid is not a constant but is determined by the activity of the water. Hence the amount of bound water found experimentally will depend on the conditions of temperature, etc., under which the experiment was made. He has attempted to correlate measurements made by different methods by taking into account the activity of the water in the system under the conditions of the measurement. He found the relation of activity to bound water content obtained by Newton and Gortner's (14) cryoscopic method for gum arabic, to be in good agreement with values derived from the vapor-pressure curve. Estimates of bound water in gelatin, agar and several other

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gels, however, made by Robinson's (18) calorimetric procedure, gave considerably higher values for the same activity than those indicated by vapor pressure measurements.

According to Briggs, measurements made on frozen gels should yield a result for bound (unfrozen) water which depends on the temperature of the frozen material but which is independent of the amount of water originally present in the gel. Jones and Gortner (8) on the other hand found, in a series of measurements made on gelatin gels by the dilatometer method, that if sufficient time was allowed for equilibrium to be established at the temperature where freezing first began, about -6°C. , no more ice appeared to be formed even when the temperature was brought as low as -50°C. They also found that the amount of water bound by the gelatin was dependent on the concentration of the gel. The relation between bound water and gel concentration was a logarithmic one from which they infer that the binding of water by gelatin is an adsorption process. To account for the fact that the amount of water remaining unfrozen was apparently independent of the temperature, they suggest that there is an increase of "adsorption pressure" as the temperature is lowered which is just sufficient to counterbalance the decrease in the vapor pressure of the ice. This is equivalent to saying that the activity of a fixed concentration of bound water varies with temperature in exactly the same way as the activity of ice.

Moran (12) observed that when gelatin gels of 12 to 40% concentration, in the form of thin disks, were frozen slowly at -3°C. , ice formation occurred entirely on the surface. The outer layer of ice could be removed from the core of partly dehydrated gel and the concentration of the latter determined. On lowering the temperature further, water passed from the unfrozen core into the outer layer of ice and the concentration of the unfrozen gel increased from 54.3% at -3°C. to a maximum of 65.5% at -19°C. corresponding to 0.53 gm. water per gram of gelatin. Moran called this the bound water and designated the water which separated between -3° and -19°C. , "interstitial" or "capillary" water. Later measurements by the same method (13) gave closely concordant results with three different samples of ash-free gelatin, for gels of various concentrations within the limits 12 to 40%, and for widely different rates of freezing. Bound water according to Moran is apparently independent both of gel concentration (within the limits stated) and of temperature (below -19°C.). Even three days' immersion in liquid air caused no further separation of ice from a sample which had been first frozen at -20°C. (12).

There are thus considerable discrepancies in the results reported for gelatin gels even when only those methods which depend on freezing the gel are considered.

Experimental

Method

The present paper gives an account of the first stages of an investigation employing the calorimetric procedure which was used by Mennie (11). Most

of the previous measurements made by this method were on such complex systems as animal tissue, etc. As Mennie has pointed out, these are of little value in estimating the water bound by the colloids present since at the temperature used (-20°C.) it is improbable that the eutectic point of the muscle juice had been reached (20) and some of the water would be prevented from freezing by the inorganic salts present. Briggs (5) has also drawn attention to this fact.

In the present work "ash-free" isoelectric gelatin supplied by the Eastman Kodak Co. (Lot No. 48) was used. The ash content actually determined was 0.04% and the moisture content was about 12%. The pH was not determined but was stated to be 4.85.

The method involves the determination of the heat required to warm a sample of gel from various initial temperatures up to the temperature of the calorimeter. All observations were corrected to the same final temperature, 25°C. , and a heat capacity curve constructed. A whole series of measurements were made on a single sample of gel which had been sealed in a container. To ensure uniform freezing conditions and make certain that equilibrium had been established, the gel was rapidly frozen in solid carbon dioxide and ether before each determination, and was then allowed to warm up to the desired initial temperature in a bath in which it was kept at constant temperature for 1 to $1\frac{1}{2}$ hr. Mennie (11) had found no evidence that repeated freezing and thawing produced any permanent change in the gel which would prevent repetition of results with the same sample. This was confirmed with a freshly prepared gel by making three consecutive runs at -78.5°C. The values obtained for the total heat capacity of container and gel were 2648.4, 2653.1 and 2644.8 cal. At the end of a long series of measurements on this sample a fourth run was made at -78.5°C. giving a value of 2635.9 cal. This is somewhat low compared with the earlier figures. However, as Barnes and Maass (4) point out, the difficulties inherent in the method of transferring the container from bath to calorimeter tend to give low results, especially at low initial temperatures. If the difference were actually due to a change in the gel it would indicate a decrease in the amount of free water, a sufficiently improbable result which is not supported by any of the other measurements on the same gel.

Calorimeter

The adiabatic calorimeter used was that designed by Barnes and Maass (3) and used by them to measure the heat capacity of ice (4). They have given a complete description of the apparatus and the method of operation. It is unique in that it employs a radiation thermel to detect differences in temperature between the inner and outer baths. This eliminates the difficulty of accounting for the heat capacity of the thermel which, in the ordinary form of adiabatic calorimeter, is immersed directly in the inner bath. It also permits of closing off the water in the inner bath from contact with the outside air, thus eliminating errors due to evaporation, etc.

The apparatus is made very sensitive to small temperature differences

between the inner and outer baths by placing the galvanometer scale about three metres away from the mirror. A deflection of 1 cm. corresponds to 0.003°C . Since a shift of 0.5 mm. on the scale can be noticed, a temperature difference of 0.00015°C . can be detected. With careful adjustment the spot of light can be kept within 1 mm. of zero on the scale so the two baths can be maintained at the same temperature within 0.0003°C .

A Beckmann thermometer was used to obtain the temperature of the outer bath of the calorimeter. It had been calibrated by the U.S. Bureau of Standards and all readings made were corrected according to the certificate supplied. The thermometer was standardized by comparing it with a standard thermometer at nine different points. Readings on the Beckmann were plotted against readings on the standard, and the straight line obtained was extrapolated to zero on the Beckmann. In this way the Beckmann zero was found to correspond to 23.65°C .

A constant-temperature bath was used to bring the sample to a known initial temperature before introduction into the calorimeter. For 0°C . a water-ice mixture and for -78.5°C . solid carbon dioxide moistened with ether was used. For temperatures between these points a large Dewar flask, filled with ether and stirred with a current of dry air, was used. The ether was brought to the required temperature by dropping in lumps of solid carbon dioxide, and the bath could be maintained at the desired temperature within 0.1°C . by the addition of small lumps of solid carbon dioxide at regular intervals.

The temperature of the bath was obtained by means of a platinum resistance thermometer which was read by the usual Wheatstone bridge arrangement using a plug-type resistance box in series with a calibrated mercury resistance of the type designed by Maass and Mennie (10), and the same galvanometer used in the thermel circuit. The platinum thermometer was calibrated by determining its resistance in melting ice, in solid carbon dioxide and in liquid air. The composition of the liquid air was determined by a gas analysis using pyrogallol as the reagent, and the corresponding temperature obtained from Claude (6). As the value for the normal boiling point of oxygen given by Claude is -182.0°C . while the International Critical Tables give -183.00°C . as the best value, 1°C . was subtracted from the temperature read from the composition. The barometric pressure was neglected as a change of 10 mm. affects the temperature only by about 0.01°C . The carbon dioxide temperature, however, was corrected for barometric pressure by Henning's formula (7).

As a check on the performance and accuracy of the apparatus, a test run on ice was made with the same platinum container which had been used by Barnes and Maass (4). The heat capacity per gram of ice between -78.5° and 25°C . was found to be 138.1 cal. as compared with 138.2 cal. obtained by Barnes and Maass.

Material

The ash-free Kodak gelatin used was in the form of sheets. The rate at which this absorbed water when immersed in it was determined. A weighed

strip of gelatin about 1 by 3 in. was immersed in water at room temperature for a definite time then quickly removed, blotted between two strips of filter paper and weighed in a covered petri dish. For gels of concentration between about 20 and 60% the desired amount of water can be introduced quite readily by immersing the gelatin in water for the predetermined time.

An amount of gelatin which would give about 20 gm. of a 24% gel was immersed in water for 10 min. It was then removed to a test tube and melted to a sol, being kept at 50° C. for 30 min., then poured into the container. At the same time duplicate portions were poured into a petri dish, weighed quickly and then dried to constant weight in an oven at 105° C. This required about 48 hr. The mean value of the duplicate determinations was 24.0% of gelatin. This was taken as the concentration of the gel.

Container for Gels

The construction of the container for the gel is shown in Fig. 1 (not drawn to scale). It was made entirely of monel metal except for a thin lead washer under the cover. Capillary spaces around the screws into which water might creep were avoided by countersinking the holes and turning the heads of the screws so that the bevelled edge fitted very exactly into the countersunk hole. The washer was of practically pure lead and was quite soft so that when the cover was screwed down firmly the joint was completely filled. The container was found to hold ether very well under a vacuum.

Two points had to be considered in filling the container. In the first place, sufficient space must be allowed for the expansion which occurs on freezing, to avoid bursting the container. In the second place, the gel should make as good contact as possible with the walls of the container in order to facilitate heat transfer and allow of equilibrium being established in the calorimeter as rapidly as possible. These requirements were met in the following manner. The sol which had been heated at 50° C. was poured into the container until the latter was about three-quarters filled. Immediately, the cover was screwed on as tightly as possible and the container rotated in a horizontal position at about 300 r.p.m. until the gel had set. Preliminary experiments showed that this caused the gel to set around the sides of the container, thus bringing into play as much surface as possible to aid in the transfer of heat, and also leaving a cylindrical space in the centre of the gel which permitted expansion on freezing. When the gel had cooled the whole was weighed. The container had already been weighed empty so the weight of the gel was determined.

The container was suspended from a cork by a silk thread of appropriate length and placed in a thoroughly dried copper tube closed at one end. The cork was sealed securely in the tube by means of adhesive tape to prevent moisture or ether creeping in. A piece of cotton wool was also introduced into

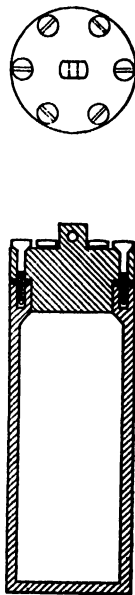


FIG. 1.
Diagram of container used for introducing gels into calorimeter.

the tube above the container as an additional precaution. Between experiments the container was always kept in a desiccator over calcium chloride to ensure that it remained dry on the outside.

The copper tube enclosing the container was placed in a carbon dioxide-ether mixture for 10 min. to freeze the gel and was then transferred to the constant-temperature bath for 1 to 1½ hr. The container was then rapidly transferred to the calorimeter and the fall in temperature observed as described by Barnes and Maass (3). At the completion of the series of runs, the gel was removed from the container and its concentration checked by drying to constant weight. The result found was 23.7%.

Results

The results obtained for the total heat capacity of the 24% gel are shown in Table I. Table II shows the heat capacity of the empty container found in exactly the same way. The heat capacity of the dry gelatin was also deter-

TABLE I
TOTAL HEAT CAPACITY OF CONTAINER AND 24% GEL

Run No.	Initial temp., °C.	Heat capacity, observed, cal.	Heat capacity, average, cal.	Run No.	Initial temp., °C.	Heat capacity, observed, cal.	Heat capacity, average, cal.
14	25.0	606.1	0.0	4	-38.3	1941.2	1941.5
15	0.0	607.8	607.0	6		1941.7	
27	-0.6*	1008.1		11	-44.5	2061.1	2062.7
28		839.3		12		2064.3	
23	-0.8	1084.9	1087.4	9	-54.2	2237.1	2234.2
24		1089.9		10		2231.3	
20	-1.3	1128.3	1129.1	7	-63.8	2393.7	2397.5
25		1129.9		8		2401.8	
18	-5.3	1291.3	1293.6	1	-78.5	2648.4	2645.6
22		1295.9		2		2653.1	
17	-15.2	1515.4	1516.7	3		2644.8	
21		1517.9		29		2635.9	
13	-27.9	1756.3	1760.0				
16		1763.7					

*Temperature of freezing point.

TABLE II
HEAT CAPACITY OF CONTAINER ALONE

Initial temp., °C.	25.0	0.0	-26.7	-57.8	-78.5
Heat capacity, cal.	0.0	-335.6	-686.4	-1077.9	-1339.5

TABLE III
TOTAL HEAT CAPACITY OF CONTAINER AND DRY GELATIN

Initial temp., °C.	25.0	0.0	-20.2	-34.9*	-56.2	-78.5
Heat capacity, cal.	0.0	-389.5	-687.0	-909.4	-1207.2	-1523.8

TABLE IV
TOTAL HEAT CAPACITY OF CONTAINER AND 87.5% GELATIN

Initial temp., °C.	25.0	0.0	-29.2	-52.4	-78.5
Heat capacity, cal.	0.0	-414.4	-900.1	-1238.6	-1613.6

TABLE V
HEAT CAPACITY PER GRAM OF GELATIN GELS

Initial temp., °C.	Dry gelatin	87.5% Gel	24% Gel	Initial temp., °C.	Dry gelatin	87.5% Gel	24% Gel
	Heat capacity in calories				Heat capacity in calories		
25.0	0.00	0.00	0.00	-5.0			-71.88
0.0	-7.18	-8.21	-22.09	-10.0			-76.38
-0.6			-40.40	-20.0	-11.36	-14.70	-82.50
-0.6			-54.14	-40.0	-15.67	-20.32	-91.49
-1.0			-61.54	-60.0	-20.76	-24.64	-99.84
-3.0			-68.54	-78.5	-24.02	-27.72	-106.30

mined. A sample was cut up into small pieces and dried in an oven at 105°C. for 24 hr. and then to constant weight in a vacuum desiccator over phosphorus pentoxide for about two weeks. The dry material was then quickly packed in the container and the latter placed in an oven at 105°C. for about six hours before screwing on the lid. The heat capacities obtained are shown in Table III. A sample of the original air-dry material was also cut into small pieces and packed in the container. The moisture content was re-determined at the same time and found to be 12.5%, corresponding to 87.5% of gelatin. The heat capacities are shown in Table IV.

The data of Tables I to IV were plotted on a large scale and smooth curves drawn to fit them. From these curves the values for the heat capacities of the samples at various temperatures were obtained by subtracting the value read from the curve for the empty container at the same temperature. Dividing the result by the weight

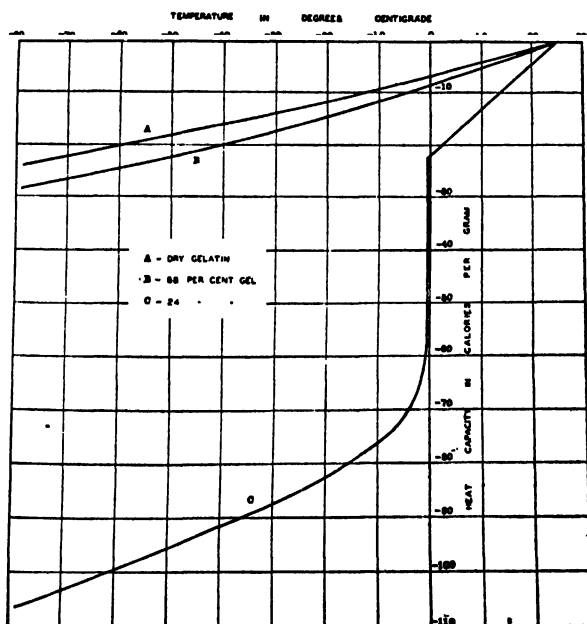


FIG. 2. Heat capacity curves for gelatin.

of the sample gave the heat capacity per gram between various initial temperatures and the final temperature of 25°C. These values are shown in Table V and plotted in Fig. 2.

The Calculation of Bound Water

Several other workers (15, 16, 17, 18, 21, 22) have made use of the calorimetric method. Thoenes (22) has given an equation for calculating bound water from calorimetric measurements which may be written:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5T_1} \quad (1)$$

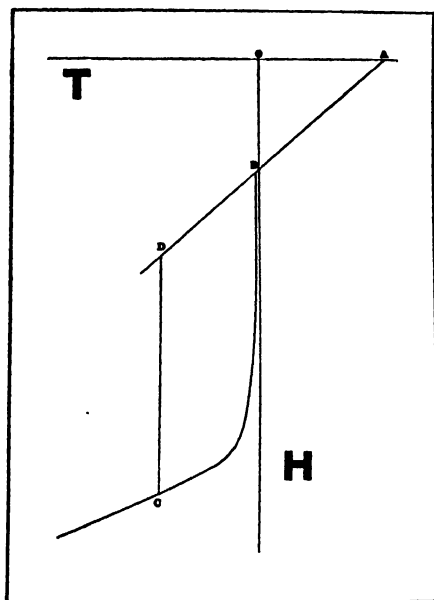


FIG. 3. Heat capacity curve illustrating Thoenes' equation.

where x is the weight of water frozen at T_1 , H is the heat absorbed when the sample is warmed from T_1 to T_2 , M is the weight of the sample and S its specific heat. The meaning of this equation may be discussed most conveniently by reference to Fig. 3. ABC is a typical heat capacity curve similar to that for the 24% gel shown in Fig. 2. The slope of AB gives the heat capacity per degree Centigrade (the quantity MS) of the unfrozen material. Extrapolation of AB to the temperature T_1 gives the point D . The heat capacity corresponding to D is evidently $MS(T_2 - T_1)$. The length of the line CD then represents $H - MS(T_2 - T_1)$. This is the heat liberated if the frozen portion of the water is melted isothermally at T_1 . The weight of water frozen is obtained by dividing this

quantity by the latent heat of fusion per gram at the temperature T_1 .

From the Kirchoff equation for the rate of change of latent heat with temperature, $\frac{dL}{dT} = (C_w - C_i)$, the latent heat at T_1 , $L_{T_1} = L_0 + T_1 \frac{dL}{dT} = L_0 + T_1(C_w - C_i)$ where C_w and C_i are the average specific heats between 0°C. and T_1 , of water and ice respectively. If C_w is taken as 1 and C_i as 0.5 and L_0 as 80 cal. per gm., $L_{T_1} = 80 + 0.5 T_1$, corresponding to Thoenes' equation.

St. John (21) derives the equation in a slightly modified form which, using the foregoing notation, would be:

$$x = \frac{H - MS(T_2 - T_1)}{80 + 0.5(T_2 - T_0)} \quad (2)$$

where T_0 is the observed freezing point of the material. From the above discussion it is evident that this modification is incorrect. The actual error in St. John's derivation was the use of 80 cal. for the heat of fusion at T_0 .

If L_{T_0} is inserted in place of 80, the equation becomes identical with that of Thoenes.

The equation is an approximation at best. The specific heats of water and of ice are not independent of temperature. The specific heat curve of water is known only to -5°C . (1, 2) and it is questionable how far below that temperature it may legitimately be extrapolated. Extrapolation to -20°C . gives a mean value of 1.03 between 0° and -20°C . Taking the recent accurately determined values of Barnes and Maass (4) for the latent heat and specific heat of ice, the equation becomes:

$$x = \frac{H - MS(T_2 - T_1)}{79.40 + 0.55T_1} \quad (3)$$

The numerator still depends on the extrapolation of the line AB (Fig. 3) *i.e.*, on assuming a constant specific heat continuing far into the metastable or unstable region below the freezing point. From the appearance of curves A and B (Fig. 2) it seems probable that the extrapolated line should bend slightly toward the temperature axis. If so, the true value of CD would be slightly greater. Hence the equation probably gives values for the water frozen which are a little low. Since the bound water is usually quite a small difference between the total water content and the portion estimated to be frozen, this equation will give values for bound water which are likely to be high. In any case the extrapolations on which it depends, even if approximately valid as low as -20°C ., the initial temperature used by Thoenes, can scarcely be relied on at temperatures any lower.

It is necessary then to find some other basis for calculation of bound water, which may be used over the whole temperature range covered by these experiments. When a gel at a temperature T_1 is warmed to T_2 , the free water which is frozen at T_1 is melted and reabsorbed into the gel. The total heat change involved in this process is independent of the path by which the process occurs. It will be the sum of the heat effects which accompany any set of intermediate steps that may be assumed. The mechanism implied in Thoenes' equation is the isothermal melting of the ice and reabsorption of the free water at T_1 followed by warming of the gel to T_2 . The possibility of a heat effect accompanying the reabsorption of the water is disregarded.

The gel might be assumed to be warmed to 0°C . without melting, the frozen portion of the water to be melted isothermally at 0°C ., the whole to be warmed to T_2 and the free water reabsorbed isothermally at T_2 . The heat capacity, H , of 1 gm. of gel between T_1 and T_2 would then be the sum of the heat effects involved in the individual steps, or:

$$H = aH_g + xH_x + (1 - a - x)H_i - \lambda. \quad (4)$$

Here H_g is the heat capacity per gram of dry gelatin, H_x is the heat capacity per gram of bound water, and H_i the heat capacity per gram of ice (including latent heat of fusion), all between T_1 and T_2 . Total heat capacities rather than specific heats are used in this equation because they are the quantities

actually measured experimentally. In Equation (4), a is the weight of dry gelatin, x the weight of bound water, and λ the heat liberated when $(1-a-x)$ gm. of water are absorbed by a gel which already contains a gm. of dry gelatin and x gm. of water. The value of λ will depend on the value of x . According to Rosenbohm (19) the heat evolved when dry gelatin takes up water is nearly proportional to the amount of water up to about 0.25 to 0.3 gm. water per gm. of dry gelatin. At this point the curve bends rather sharply and the heat change accompanying further imbibition of water is small. Rosenbohm estimates that the entire heat of swelling is liberated when 0.5 gm. water has been taken up per gm. of dry gelatin. Moran (12) estimates the bound water to be 0.53 gm. per gram dry gelatin and a similar figure was obtained by Mennie (11) from earlier calorimetric measurements, using Thoenes' equation. If these estimates are correct, λ in the above equation should be zero or negligible.

Omitting λ , the amount of bound water is then given by

$$x = \frac{aH_g + (1-a)H_i - H_{obs.}}{H_i - H_x} \quad (5)$$

H_g , the heat capacity of the dry gelatin, is obtainable from the data of Table V. Values of H_i are given by Barnes and Maass (4). It is obvious that the specific heat of bound water cannot be the same as that of free water. A value must be found for H_x . An estimate is possible from the data of Table V.

TABLE VI
HEAT CAPACITY PER GRAM OF BOUND WATER IN 87.5% GELATIN

Temp., °C.	25.0	0.0	-3.0	-5.0	-10.0	-20.0	-40.0	-60.0	-78.5
Heat capacity, cal.	0.0	-20.8	-23.1	-24.6	-28.3	-35.3	-46.7	-53.4	-58.4

TABLE VII
BOUND WATER PER GRAM OF DRY GELATIN IN 24% GEL

Temp., °C.	-3.0	-5.0	-10.0	-20.0	-40.0	-60.0	-78.5
Bound water							
Equation (5)	0.69	0.57	0.46	0.37	0.33	0.26	0.24
Equation (3)	0.82	0.70	0.61	0.56	—	—	—

In an 87.5% gel it is to be expected that the water is all bound. This is confirmed by the absence of any break in the curve (Curve B, Fig. 2). Subtracting the heat capacity of 0.875 gm. of dry gelatin should then give the heat capacity of 0.125 gm. of bound water. Table VI gives values per gram of bound water calculated in this way from the data taken from the smooth curves (A and B) of Fig. 2. It must be pointed out that if there are different stages or degrees of binding, the specific heat of 1 gm. of bound water in an 87.5% gel is not necessarily the same as the specific heat of 1 gm. of bound water in a 24% gel. It is probable however that any difference would be insignificant.

Values for bound water calculated by means of Equation (5) are given in the second line of Table VII. In the third line are shown for comparison values calculated by Thoenes' equation (Equation 3). The latter agree closely with those previously obtained in the same way by Mennie (11) and also with Moran's work (13). The figures in the second line are markedly lower. They do not appear to reach a minimum value at $-20^{\circ}\text{C}.$ as Moran found, but continue to decrease with the temperature as predicted by Briggs (5). It has been shown already that Thoenes' equation tends to give too high an estimate of the bound water. Nevertheless, the figures in the second line of Table VII require further confirmation, and work is in progress to this end. The values for the heat capacity of bound water in Table VI were derived from a relatively small difference between the experimental values of Curves *A* and *B* (Fig. 2) and hence may be subject to a considerable experimental error. Accordingly, further measurements are planned on very concentrated gels. Additional measurements are also being made on more dilute gels of different concentrations and at temperatures even lower than $-78.5^{\circ}\text{C}.$

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INFLUENCE OF SOIL TEMPERATURE AND SOIL STERILIZATION ON THE REACTION OF WHEAT SEEDLINGS TO *OPHIOBOLUS GRAMINIS* SACC.¹

BY A. W. HENRY²

Abstract

In this investigation Marquis wheat seedlings reacted similarly to a virulent Alberta strain of *Ophiobolus graminis* in sterilized and unsterilized soil at low temperatures, but differently at higher temperatures. At 13° C. for instance, the blighting was about equally severe in sterilized and unsterilized soil, but at 27° C. most of the seedlings were killed in the sterilized soil, while those in the unsterilized soil were only slightly attacked. The protective value to wheat of the constituents of unsterilized soil against soil-borne inoculum of *Ophiobolus graminis* appears, therefore, to vary with temperature, being under the conditions of these studies relatively slight at soil temperatures below 20° C.

Introduction

In most investigations on the effect of soil temperature on the development of plant diseases, conducted under controlled conditions, sterilized soil has been used. It seemed that the conclusions drawn from such studies might not always apply under field conditions, where the soil usually is not sterilized. Therefore, in these studies on the effect of temperature on the development of foot-rot diseases of wheat, it was decided to use both sterilized and unsterilized soil, so that the results might be compared. The data reported in this paper apply only to studies made with *Ophiobolus graminis*.

Review of the Literature

The influence of temperature on the growth of *Ophiobolus graminis* in artificial culture has been investigated by Davis (1), who found that growth occurred between 4° and 33° C. Three strains were studied. The optimum temperatures for the growth of one strain originating in New York, ranged between 19° and 24° C. and for two others from Oregon and Arkansas, between 23° and 24° C. The effect of temperature on the development of the "take-all" disease of wheat was studied at the University of Wisconsin by McKinney and Davis (6). They found that the optimum temperature range for the disease in the case of wheat seedlings was considerably lower than for the vegetative growth of the parasite, being between 12° and 16° C. Their studies were made in sterilized soil maintained at constant temperatures. The inoculum used was produced by growing the fungus on a mixture of cooked oat and barley kernels. Commenting on these studies, Jones, Johnson and Dickson (4) point out that the apparent temperature optima may shift somewhat, regardless of variations in moisture content, thus indicating that some other undetermined factors not controlled by corresponding temperature or moisture variations may have a decided influence on the occurrence of this disease. McKinney (5)

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has also observed that *Ophiobolus graminis* often "behaves in a very perplexing way at times in regard to both fruiting and infection."

Experimental Methods

The pathogenic fungus used in these studies was a virulent strain of *Ophiobolus graminis* (No. IV) isolated in this laboratory by Mr. F. R. Davies*, from wheat collected in 1930 at Athabasca in northern Alberta. The medium selected for the production of inoculum consisted of typical black soil of the Edmonton district plus 10% of corn meal. The fungus grew well on this medium and caused severe damage to wheat seedlings when added to the soil. The inoculum was produced in small Erlenmeyer flasks, each containing 50 gm. of the sterilized soil-cornmeal mixture plus 27 cc. of distilled water. The fungus was allowed to develop at room temperature on this medium for about three weeks, when the contents of each flask were added at seed level to a 6-in. pot of soil. Half of the pots of soil had been sterilized for four hours at 15 lb. pressure, while the other half had not been sterilized. Marquis wheat kernels previously surface sterilized with a formaldehyde solution (1:320) and washed with water, were then sown at the rate of 25 kernels per pot and covered with about an inch of soil. The pots were then embedded in moist sand in the cans of soil-temperature tanks of a type similar to those in use at the University of Wisconsin (4). In each tank there were four pots of unsterilized soil, three pots of sterilized soil which received inoculum and one pot of sterilized soil to which no inoculum was added, and which served as a check. The four temperature tanks available were maintained at average soil temperatures of 13°, 18°, 23° and 27° C. respectively, for a period of 25 days, when data were taken on the disease reaction of the wheat seedlings.

Results

At the conclusion of the experiment, the following notes were taken on each seedling: height in cm., degree of blighting of the shoot, degree of rotting of the basal part of the stem

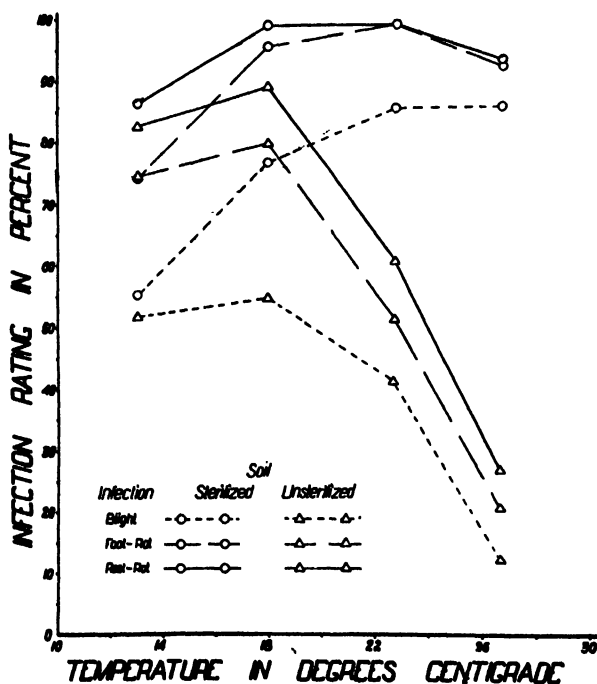


FIG. 1. Reaction of Marquis wheat seedlings to *Ophiobolus graminis* at different soil temperatures in sterilized and unsterilized soil.

* Graduate assistant in Plant Pathology.

or foot, and the degree of rotting of the roots. The three latter notes were taken according to the system used by McKinney and Davis (6), the average figures being recorded as percentage. The summarized data are shown in Table I and illustrated graphically in Figs. 1 and 2. The actual effect on the seedlings of the different treatments is shown in Plate I.

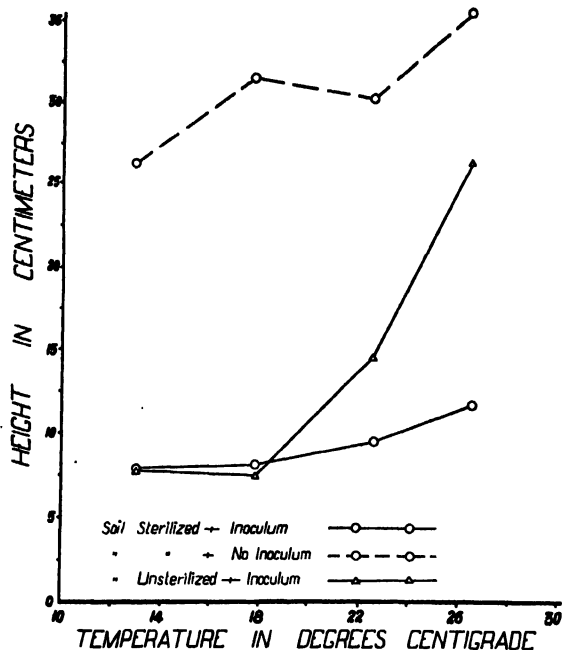


FIG. 2. Effect of *Ophiobolus graminis* on the height of Marquis wheat seedlings in sterilized and unsterilized soil kept at different temperatures.

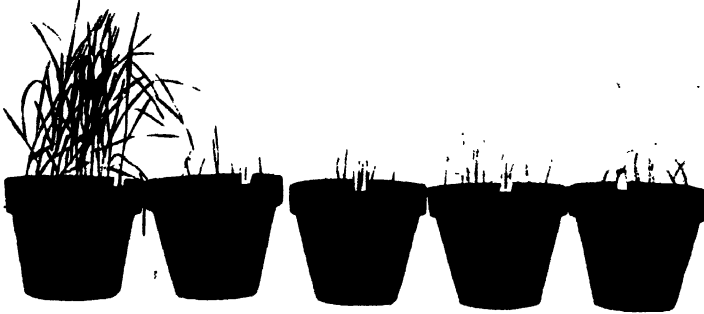
whereas those in the unsterilized soil were almost as vigorous as the uninoculated ones of the check.

TABLE I
REACTION OF MARQUIS WHEAT SEEDLINGS TO *Ophiobolus graminis* (No. IV) AT
DIFFERENT SOIL TEMPERATURES, IN STERILIZED AND UNSTERILIZED SOIL

Notes		Sterilized soil				Unsterilized soil			
		13°C.	18°C.	23°C.	27°C.	13°C.	18°C.	23°C.	27°C.
Height of plants, cm.	Inoculated	7.9	8.0	9.4	11.6	7.8	7.3	14.4	26.1
	Check	26.1	31.4	30.2	35.4	—	—	—	—
Blight rating, %	Inoculated	55.3	76.5	85.6	85.7	51.8	54.9	40.3	12.2
	Check	0.0	0.0	0.0	0.0	—	—	—	—
Foot-rot rating, %	Inoculated	74.2	95.5	98.9	92.1	74.4	79.7	51.9	20.3
	Check	4.0	0.0	2.0	0.0	—	—	—	—
Root-rot rating, %	Inoculated	86.5	99.0	98.9	93.2	82.5	89.0	60.6	26.6
	Check	0.0	0.0	0.0	0.0	—	—	—	—



13°C.



18°C.



23°C.



27°C.

UNSTERILIZED SOIL

STERILIZED SOIL

*Relative amount of blighting of Marquis wheat seedlings in sterilized and unsterilized soil kept at different temperatures, following inoculation with *Ophiobolus graminis*.*

The effect on the development of the disease may best be observed by an examination of Fig. 1. If the curves are examined in pairs representing blight, foot-rot, and root-rot respectively, it will be noted that in each case the trend of the curve for sterilized soil is quite different from that for unsterilized soil. It would appear that the effect of temperature was overshadowed by some other factor or factors in the unsterilized soil, at the higher temperatures.

It may perhaps be wondered why no checks on unsterilized soil were included in the experiment. This was simply because of lack of sufficient space. However, in experiments conducted since, with the same and similar soils, both kinds of checks have been used and no differences of importance have been observed between them. The above experiment has also been repeated with almost identical results.

Discussion

As to the discrepancy between the results in sterilized soil and those in unsterilized soil, the writer has previously suggested a possible explanation (3), namely, that meteorological factors may influence the development of the natural soil microflora, which in turn may affect the development of the pathogene and the disease caused by it. Thus it would only be necessary to assume that certain antagonistic saprophytes in the soil, several of which have been reported by Sanford and Broadfoot (8), are more active in inhibiting *Ophiobolus graminis* at relatively high temperatures and have little effect at temperatures below 20° C. It should be noted in this connection, however, that Jones, Johnson and Dickson (4) in their extensive summary of the effect of soil temperature on the development of plant diseases caused by soil-borne pathogenes, have also pointed out the possibility "that any variations in soil environment, in addition to their direct relations on host and parasite, may have some influence upon the general development of the soil flora and fauna which may in turn indirectly affect the phytopathological results." In general, however, they considered that this was not a matter of primary importance. Field results from plantings made on different dates, and therefore exposed to different temperatures, in several instances checked quite closely with those from the more exact greenhouse experiments. In the case under consideration, however, had only sterilized soil been used, it might have been concluded that severe seedling injury would occur under natural conditions at soil temperatures as high as 27° C., whereas from the results in unsterilized soil, this does not seem probable. It is possible of course that the plants in unsterilized soil at 27° C. might have succumbed later had the experiment been prolonged, but from the amount of lesioning present and from the condition of the plants, this hardly seems likely.

Although attempts to produce the disease in the field by applying artificial inoculum to the soil have frequently failed, the disease does occur in severe form under natural field conditions. Its severity, however, fluctuates widely in different districts and from season to season. Evidently the proper balance between the various factors necessary for severe infection occurs only occasionally. From the results reported, it appears that low soil temperatures

would be especially important in the production of severe infection under field conditions because of the fact that the soil is not sterilized, but if the soil were sterilized, low temperatures would not be necessary for severe infection with the strain of *Ophiobolus graminis* used in these studies.

It is possible that the results presented may help to explain the difference in severity of "take-all" of spring wheat in different geographical regions. For instance, observations made during the past ten years indicate that "take-all" is much more destructive to spring wheat in western Canada than in the United States. In fact, the disease has only recently been found in spring wheat in the United States according to Fellows and Hungerford (7) who report a severe outbreak in southern Idaho in 1930. It has been known in western Canada, on the other hand, since 1923 (2) and is now considered one of our most destructive diseases of spring wheat. These observations may possibly be explained by the fact that soil temperatures in western Canada are, at least at times, appreciably lower during the growing season than they are in the spring-wheat regions farther south in the United States. In the case of winter wheat the situation is somewhat different. Severe damage occurs in the United States as well as in western Canada, though the amount of winter wheat grown here is relatively small. However, in both countries, winter wheat is normally exposed during part of its life to low soil temperatures, and it may be at some time during this period that infection chiefly takes place.

It is realized that, besides those mentioned, there are probably other important factors concerned in determining the severity of infection caused by soil-borne inoculum of *Ophiobolus graminis* and other pathogenes. The amount of inoculum present, for instance, may be one of them. However, if we confine our attention to the factors discussed above, namely, soil temperature and the activities of the saprophytic organisms of the soil, the situation is sufficiently complex to make it unsafe to draw general conclusions for all soil-borne pathogenes. What might apply to one might not apply to another. It does not necessarily follow, for example, that the soil saprophytes which are antagonistic to *Ophiobolus graminis* affect other pathogenes in a similar manner. It is believed, however, that it has been demonstrated in at least one instance that disease reaction may differ markedly in sterilized and unsterilized soil at certain temperatures, and be approximately the same at other temperatures. In view of this, and because of the fact that field soils usually are not sterilized, it would seem desirable, in studying the influence of soil temperature on plant diseases, to at least check the results of experiments performed with sterilized soil, by repeating the experiments using the same soil without sterilizing it.

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VEGETATIVE PROPAGATION OF WILD OATS, *AVENA FATUA*, AND OTHER ECONOMICALLY IMPORTANT SPECIES OF *AVENEAE* AND *HORDEAE*¹

BY L. E. KIRK² AND T. K. PAVLYCHENKO³

Abstract

Following the discovery that small sections of wild oats seedlings could become rerooted and develop into normal plants, a study was undertaken to determine from what part or parts of the seedlings new growth can originate, and whether cultivated oats, wheat, barley and spring rye, would behave in a similar manner. Special attention was given to the problem of vegetative propagation in wild oats as a factor in the control of this weed.

Small sections of wild oats seedlings, one inch in length and containing the coleoptile node, became rerooted under favorable conditions and produced fully developed plants. Cultivated oats behaved in a similar manner to wild oats in this respect, but the latter produced the more vigorous growth.

When land is infested with wild oats and is plowed shallow, or cultivated shortly after the seedlings have emerged from the soil, a considerable proportion of them, under certain conditions, may produce new plants by vegetative regrowth. The extent to which this may occur in the field depends largely on the soil moisture as well as on other conditions which facilitate rerooting, such as shallow plowing, and packing when conditions for plant growth are favorable.

With young seedlings of wild oats at time of emergence, regrowth occurred mostly from a small area located between ground level and one inch below the surface. The same was found to be true of cultivated oats. With seedlings of wheat, barley and spring rye, at time of emergence, regrowth originated only from nodal tissue close to the seed. The difference in this respect, between oats, *Aveneae*, on the one hand, and wheat, barley and rye, *Hordeae*, on the other, is due to the fact that the area of elongation in oats is the mesocotyl, whereas in other cereals it is the first internode.

At later stages in seedling development of both oats and the other cereals, nodes which were capable of regrowth developed immediately below and above the ground level. With older seedlings in the first-, second-, and third-leaf stage, the youngest node above ground, which is the one nearest the soil, had the greatest power of regrowth.

Introduction

Weeds have become a serious problem in western Canada. They constitute a factor that has persistently forced upward the cost of production. About three years ago the feeling became general among leading agriculturists that more definite action should be taken to combat the weed menace, and this feeling became intensified when depressed prices of farm products made urgent the need for decreased costs of production.

It was decided that a general campaign of education and research work on weeds should be initiated. Existing methods of weed control were, for the most part, based upon general observations. It was believed that more effective methods of control for the different weeds could be developed if

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precise information on the outstanding biological features of each weed species was available. As a part of the weed campaign, a research program was initiated at the University of Saskatchewan in 1930, with the co-operation and financial assistance of the National Research Council of Canada, for the purpose of making fundamental biological studies on the most important weed species. A prominent place in these investigations was given from the beginning to wild oats, since this is one of the most serious weeds in the grain growing areas.

In 1930, during the course of an experiment on the effect of weathering on the germination of wild oats seeds in soil, it was accidentally observed that seedlings could be cut in two and that some of the cut portions would produce new roots and grow into normal plants.

Soil and wild oats seeds were thoroughly mixed and placed in boxes 7 in. deep with wire screen bottoms, and buried to soil level. These boxes were to be brought in at different dates, one at a time, and germinated to determine how many of the seeds at different depths, which did not germinate at once, would grow later.

After one month of weathering in the ground, one box was brought in, the soil in it cut horizontally into seven one-inch layers by means of a special device, and the number of germinated seeds in each layer was recorded. Those seeds in each layer of soil which had not germinated were carefully sorted out, counted, mixed again with the soil of the layer in which they had been found, and placed in separate trays to be observed for a longer time in a greenhouse. At this time most of the seeds at all depths had germinated. Some seedlings thus produced had emerged above the surface while others, especially those at the lower depths, were only partly grown. Since the soil was cut into layers, all the seedlings were cut into one-inch sections, as shown in Fig. 1. Each layer of soil contained, therefore, not only a known number of ungerminated seeds, but also cut portions of the seedlings.

A germination count was made 14 days later when it was found that a few more seeds had grown, but the number of new plants exceeded the number of seeds. In the top layer of soil only 23 seeds were left, but 88 new plants had emerged. The numbers were not so large from the lower layers, but there was still a disproportionate number of seedlings. An examination of the root system of the plants showed that the majority of them had grown from the small sections of the seedlings left in the soil. Several of these vegetatively reproduced plants were transplanted into nine-inch flower pots and allowed to grow to maturity. Fig. 2 shows one of these which developed an extensive root system, attained a height of 42 in., produced four normal tillers, and matured seeds.

The discovery that cut sections of wild oats seedlings could establish themselves as new plants at once suggested that the same phenomenon might be of common occurrence in the field, and that it might be of practical importance in connection with the control of this weed. It also indicated a problem of considerable theoretical interest.

Literature Review

Vegetative propagation in perennial grasses has been extensively studied by forage crop workers, horticulturists and greenkeepers all over the world. References to this subject in the literature are very numerous. Perennial grasses, possessing various types of underground stems and bearing numerous overwintering buds, are easily propagated by small sections of their subterranean parts. The vegetation which results from such cuttings is as vigorous as that produced from seed.

An entirely different situation exists with regard to the annual grasses. The only cases where the writers were able to find even indirect indication as to the possibility of vegetative regrowth among these are given below.

Percival (1) refers to Miller's experiment in which tillers were separated from the mother wheat plant, together with their adventitious root system; these, when transplanted, grew to maturity. Shirreff (1) repeated Miller's experiment with still better results. In 1908 and 1909 Demchinsky considerably increased the yield of a single wheat plant by covering the stems above ground with soil in order to stimulate tillering. Tillers of several orders were produced in succession. In 1928 a very interesting experiment was conducted by Simmonds (2, 3). He studied the effect of severing the seminal roots and loss of the whole underground part below the crown node, on the further development of the injured plant. He concluded that "seminal root amputations and subcrown internode cuts cause weak tillering and delayed ripening." The plants grew, but were somewhat retarded in their development by the injury.

It will be well to keep in mind, however, that in all these cases the parts which continued to grow had all the principal organs of a normal plant, that is, roots, leaves and stems, established and well developed before they were detached from the mother plant. In Demchinsky's case, the tillers were not even detached from the original seed plant. In no case could it be considered that the authors were dealing with material produced by means of vegetative cuttings.

Scope of the Work

Wild oats, cultivated oats, wheat, spring rye and barley were chosen for experimentation. These include the main economically important annual species of *Aveneae* and *Hordeae* in this country. Experiments were carried on both in the greenhouse and under field conditions for the purpose of securing answers to the following questions:

1. Which particular parts of the seedlings of wild oats are capable of vegetative propagation?
2. What particular stages of plant development are most favorable for regrowth?
3. Are wild oats plants peculiar in this respect or do cereal crop plants behave in a similar manner?
4. To what extent are wild oats propagated vegetatively in the field, and how is regrowth related to the control of this weed?

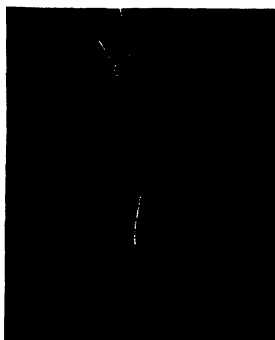
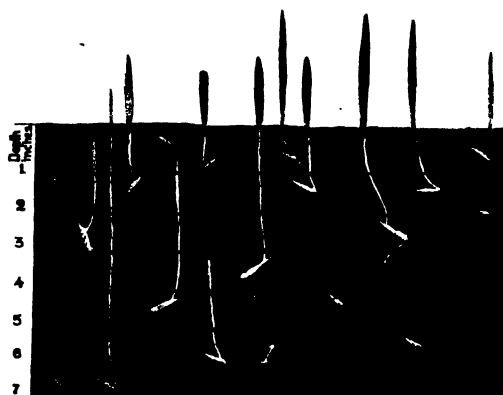


FIG. 1. Method of cutting the soil which resulted in severing the wild oats seedlings into one-inch sections. FIG. 2. Specimen of wild oats grown from a one-inch section of a young seedling. FIG. 3. Wild oats plant propagated vegetatively from the uppermost one-inch section cut from a seedling before emergence, excavated 18 days after planting. FIG. 4. Wild oats plants produced vegetatively from one-inch sections of seedlings (from left to right) cut on the first, fourth, sixth, tenth and thirteenth day after emergence. In each case the section included the top and 1 in. of the underground stem. FIG. 5. Wild oats plant produced vegetatively from a one-inch section of a seedling four days after emergence, taken at a depth from 2 to 3 in. below the surface. FIG. 6. Wild oats plant produced vegetatively from the stem cut below the first node above the soil level. The cutting was planted on the thirteenth day after emergence and excavated on the twenty-fourth day.

A. Vegetative Propagation in Wild Oats, *Avena Fatua*

1. Regrowth from One-inch Sections of Wild Oats Seedlings Cut Before Emergence

Wild oats seeds were planted in greenhouse flats 7 in. in depth. Just before emergence, 50 seedlings were carefully separated from the soil by removing one side of the box and breaking away the earth. The seedlings were then cut into one-inch sections, beginning from the top, and all those of the same depth were transplanted into separate flats and covered with $\frac{1}{2}$ in. of soil. In every case the top portion was designated "Section 1." Those taken at depths of 1 to 2, 2 to 3, 3 to 4, 4 to 5, 5 to 6, and 6 to 7 in. were designated Sections 2, 3, 4, 5, 6 and 7 respectively. Care was taken not to use plant material containing adventitious roots developed before the seedlings were cut into sections in order to avoid confusion, on the one hand, between genuine vegetative regrowth, where all parts of the re-established plant came from tissues of the cut seedlings, and on the other, normal tillering, where shoots developed before they became detached from the seed-piece. Fig. 3 shows a normal plant developed from Section 1. The data secured from this experiment are shown in Table I.

TABLE I

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS OF WILD OATS SEEDLINGS CUT BEFORE THEY EMERGED ABOVE GROUND. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.*
No. of cuttings survived	11	2	0	0	0	0	0
Height, in.	8	6	0	0	0	0	0
Per cent survived	22	4	0	0	0	0	0

NOTE:—Depth of seeding, 7 in. Records taken 40 days after the plants were sectioned.

*This section included the seed and seminal roots.

The results from this experiment indicate that:

1. A large number of one-inch sections of wild oats seedlings cut before emergence developed new root systems and stems, and grew as normal plants.
2. Regrowth was confined to portions of seedlings situated from 0 to 2 in. in depth.
3. That portion of the seedling situated from 0 to 1 in. in depth was the one which produced the majority of new plants.
4. Sections taken at a depth of 1 to 2 in. produced new plants in only a few cases. As a rule these were less vigorous than those developed from sections taken at a shallower depth.

2. Regrowth from One-inch Sections of Wild Oats Seedlings Grown at Different Depths and Cut One Day After Emergence

In this experiment seeds were planted at depths of 5, 6 and 7 in. The seedlings were cut one day after emergence, and they were approximately three days older than those used in the preceding experiment. Section;

TABLE II

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS OF WILD OATS
SEEDLINGS GROWN AT 5, 6 AND 7 IN. IN DEPTH, AND CUT ONE DAY AFTER
EMERGENCE. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.
<i>Seeded 5 in. deep</i>							
No. of cuttings survived	45	2	2	0	0		
Height, in.	13	13	8	—	—		
Per cent survived	90	4	4	0	0*		
<i>Seeded 6 in. deep</i>							
No. of cuttings survived	19	3	1	1	0	0	
Height, in.	12	10	6	6	—	—	
Per cent survived	38	6	2	2	0	0*	
<i>Seeded 7 in. deep</i>							
No. of cuttings survived	17	0	1	0	0	0	0
Height, in.	11	—	—	—	—	—	—
Per cent survived	34	0	2	0	0	0	0*

*The lowest section in each case included the seed and seminal roots.

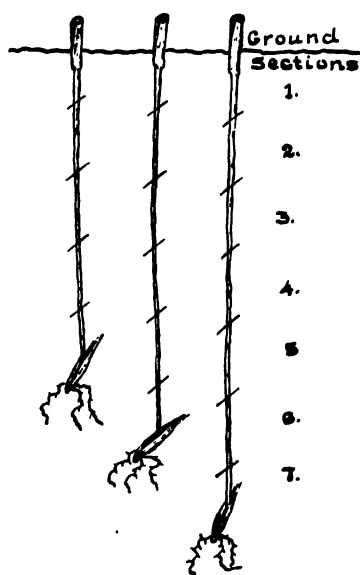


DIAGRAM 1. Method of cutting sections in Experiments Nos. 1, 2 and 3.

therefore, included 1 in. of the underground stem and also the above-ground portion (see Diagram 1). All other sections were 1 in. in length. The results obtained in this experiment are shown in Table II.

The results obtained from this test seemed to indicate that a difference in depth of seeding did not appreciably affect the location of that portion of the underground stem which was capable of regrowth after being cut into one-inch sections. At all three depths of seeding, only Section No. 1 grew readily. In a few cases, Sections Nos. 2 and 3 re-established themselves as new plants, but the number of these was very small. Considering the fact that, in the first experiment, none of the sections below 2 in. grew, while in this test, where the plants were about three days older, they did so, it might be thought that the age of seedlings had some effect upon the activity of the meristematic tissue of sections located at the lower depths.

Otherwise, the results from this experiment are in good agreement with those obtained in the previous test.

3. Effect of Age of Seedlings on the Number of New Plants Produced from Cut Sections

This experiment was made to determine how the particular stage of plant development would affect the amount of regrowth. Seeds were planted 7 in. deep, but the cuttings were made 1, 4, 6, 10 and 13 days after emergence. The material was handled as described in the preceding experiments.

The results are shown in Table III, and a few typical plants are shown in Figs. 4 and 5. The method of cutting sections was similar to that shown in Diagram 1.

TABLE III

NUMBER OF NEW PLANTS PRODUCED FROM ONE-INCH SECTIONS CUT FROM SEEDLINGS OF DIFFERENT AGES. FIFTY CUTTINGS TRANSPLANTED IN EACH SECTION AT EACH AGE

Description of sections	0 to 1 in.	1 to 2 in.	2 to 3 in.	3 to 4 in.	4 to 5 in.	5 to 6 in.	6 to 7 in.
<i>One day after emergence</i>							
No. of cuttings survived	46	1	0	0	0	0	0
Height	13	6	—	—	—	—	—
Per cent survived	92	2	0	0	0	0	0
<i>Four days after emergence</i>							
No. of cuttings survived	10	0	0	0	0	0	0
Height	8	—	—	—	—	—	—
Per cent survived	20	0	0	0	0	0	0
<i>Six days after emergence</i>							
No. of cuttings survived	9	0	0	0	0	0	0
Height	11	—	—	—	—	—	—
Per cent survived	18	0	0	0	0	0	0
<i>Ten days after emergence</i>							
No. of cuttings survived	13	0	0	0	0	0	0
Height	14	—	—	—	—	—	—
Per cent survived	26	0	0	0	0	0	0
<i>Thirteen days after emergence</i>							
No. of cuttings survived	27	2	0	0	0	0	0
Height	15	7	—	—	—	—	—
Per cent survived	54	4	0	0	0	0	0

NOTE:—Records taken 40 days after plants were sectioned.

Fig. 4 shows plants which were grown from the top sections, including 1 in. of the underground stem, cut from seedlings 1, 4, 6, 10 and 13 days after emergence. These were carefully selected so as to represent fairly the plants grown in each series. Starting from left to right one may see that the first specimen (1 day after emergence) showed vigorous top and root development. The second plant (4 days after emergence) was somewhat inferior in these

respects. Specimens 3 and 4 (6 and 10 days after emergence) were very weak as compared with the two preceding ones. Specimen No. 5 (13 days after emergence) exhibited again a well-developed root system and top growth.

The difference in behavior of cut portions taken from material of different ages was so striking that it was found necessary to repeat the experiment. In both cases, however, the results were practically the same. Microscopical examination of the seedlings of different ages showed that the nodal tissue of a plant one day after emergence had hardly become differentiated sufficiently to be distinguished from ordinary stem tissue. At the same time, the outermost stem tissue surrounding the nodes at this stage was very tender. Four days after emergence, the nodes were quite apparent, but while the outermost layers of cells indicated further progress in differentiation, they were still tender and soft. In six- and ten-day material, the secondary roots could be observed starting at the nodal region inside the stem. The outermost tissue of the stem at this stage had hardened sufficiently to exert considerable resistance to the secondary roots pushing through it. The secondary roots of seedlings 13 days after emergence, in nearly all cases, had already emerged from the stem tissue in the form of tiny protuberances around the nodes.

When very young seedlings were cut into small sections, growth appeared to be active in the meristematic tissue of the nodes. The meristem of the young seedlings, being in a very active stage of cell division, differentiated rapidly into stem and roots. These latter easily pushed through the tender tissue of the outermost layers of the stem, reached the soil, and began to support the new growth. Nodes of plants which were sectioned four days after emergence, had, for the most part, passed beyond this stage of cell division, and moreover, the external layers of the original stem tissue seemed to offer much greater resistance to the new roots pushing through them than they did at an earlier stage. These are, perhaps, the two main reasons why the majority of cuttings taken from such material were dead before new roots had developed.

The secondary roots of seedlings older than 10 days after emergence were well advanced in their development at the time of cutting, and usually continued to grow after being detached from the mother plant. For this reason, sections cut from seedlings older than 10 days after emergence re-established themselves nearly as readily as those taken from seedlings less than 4 days old. Sections taken at greater depths as a rule died, but occasionally one of them survived and produced a healthy plant (see Fig. 5).

The results of the experiment to determine the effect of age of seedlings on vegetative regrowth in wild oats may be summarized as follows:

1. The largest number of plants and the strongest were obtained from sections of young seedlings up until 4 days after emergence.
2. Seedlings which were sectioned 4 to 10 days after emergence gave fewer and weaker plants than younger seedlings.
3. Sections cut 10 to 13 days after emergence produced a higher percentage of plants than those from 4 to 10 days.
4. In all cases, sections of the stem nearest the soil surface produced the greatest number of new plants.

4. Regrowth from the Stem Above Ground

In preceding experiments it was evident that vegetative regrowth occurred readily from one-inch sections cut from seedlings of different ages. They showed also that regrowth is most likely to occur from the part which is located in the first inch below the surface. There was not sufficient information, however, to indicate whether new plants could be produced from sections of seedlings which did not contain nodal tissue, that is, from internodes, though of course there was every reason to believe that this would not be possible. The point could not be decided from the preceding experiments for the reason that nodal tissue had not been sufficiently differentiated so that it could be observed at the time when plants were sectioned. Definite information was obtained on this point in this and other experiments, which showed that nodes were the only regions from which both new roots and stems could originate.

Because it was not possible to locate the nodes in very young seedlings, it was decided to use older plants from which sections of the stem above ground could be taken, as it was found that the nodes could be identified at any time after the second leaf made its appearance. With such material it was possible to plant cuttings in such a way that only internodal tissue was buried under the ground. In other cases, they were planted with at least one node covered with soil.

Three series of plants were grown in this experiment, each sectioned at different stages of development as shown in Diagram 2, the experiment being done in duplicate. In one case the top portions were transplanted so that only the internode was covered with soil, while in the other case, corresponding top portions were planted so that the bottom node in each case was placed under the surface.

Data on the number of sections which became re-established as new plants were recorded at intervals of 20 days after they were sectioned.

The data from the 300 sections which were planted so that only the internode was buried are not tabulated as none of them grew, but those sections

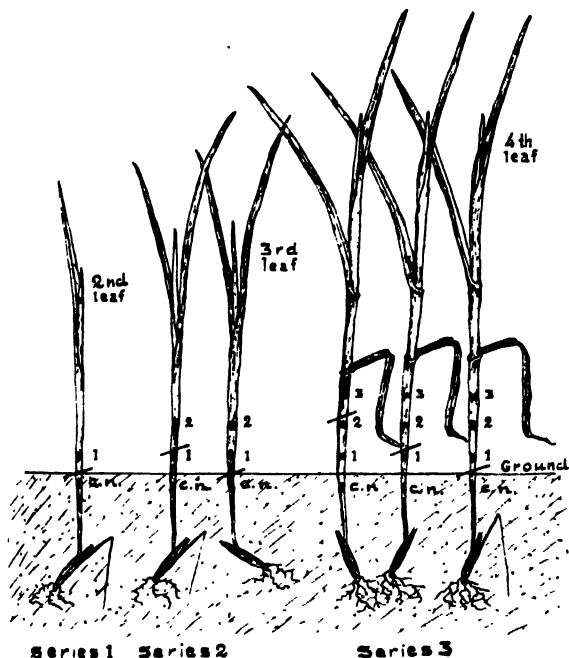


DIAGRAM 2. Method of sectioning wild oats seedlings at different stages of growth in relation to the nodes above ground (c.n. = crown node).

which were planted with their lowest nodes covered with soil, in many cases re-established themselves as new plants (see Table IV). This is substantial evidence that regrowth occurs only from nodes and not from internodes. Fig. 6 shows a plant produced from one of these cuttings.

TABLE IV
NUMBER, HEIGHT, AND PER CENT OF NEW PLANTS PRODUCED VEGETATIVELY FROM THE TOP PORTIONS OF WILD OATS SEEDLINGS CUT OFF BELOW THE 1ST, 2ND, AND 3RD NODES ABOVE GROUND, AND PLANTED WITH THE LOWEST NODE BURIED UNDER THE SURFACE

Series	Position of node above ground	No. of tops planted	No. of plants survived	Height, in.	Per cent of plants survived
1 1st node above ground. Section cut on the day when the second leaf appeared	1st	50	38	13	76
2 1st and 2nd nodes above ground. Section cut on the day when the 3rd leaf appeared	2nd 1st	50 50	3 12	11 16	6 24
3 1st, 2nd and 3rd nodes above ground. Section cut when the 4th leaf appeared	3rd 2nd 1st	50 50 50	0 2 11	— 14 18	0 4 22

NOTE:—Records taken 40 days after plants were sectioned.

A closer examination of the data presented in Table IV shows that in each series it was the first node above ground and the last to emerge that produced the largest number of new plants. Older nodes higher up on the stem gave very few plants. It was also apparent that the material taken from the youngest plant, having only one node above ground, rerooted much more readily, and produced three times as many plants as that secured from older seedlings, which had two or three nodes above ground.

Field Experiments

From experimental work on the problem of regrowth of wild oats under greenhouse conditions it is obvious that this weed may be propagated vegetatively. Thus, under certain conditions, there is the possibility that plants may re-establish themselves after having been plowed down or cultivated. The phenomenon, therefore, may be one of considerable economic importance. For the purpose of studying this particular problem, certain tests were undertaken under field conditions throughout the season of 1931. Owing to the fact that the season was extremely dry, it was found necessary to water the soil before the cuttings were transplanted. When the heat was very great, it was found useful to protect the newly planted cuttings by shading them with wrapping paper for one or two days. Even these precautions did not furnish conditions nearly as favorable as those which occur in the spring, when regrowth is most likely to occur under field conditions.

TABLE V

NUMBER OF NEW PLANTS PRODUCED FROM THE TOPS OF WILD OATS SEEDLINGS CUT OFF AT DIFFERENT STAGES OF PLANT DEVELOPMENT AND PLANTED IN THE FIELD, 10 SEEDLINGS BEING USED IN EACH CASE

Type of sectioning	Days from emergence											
	1	1	1	2	3	4	5	12	13	21	25	32
	Number of re-established plants											
Tops cut at soil surface	0	0	0	0	1	1	0	0	1	2	1	0
Tops cut 1 in. below surface	10	10	10	10	9	9	4	10	8	9	10	10

5. Vegetative Propagation of Wild Oats in the Field, from Sections Cut at Various Stages of Plant Development

Wild oats seeds were planted in the field in eight-foot rows at a depth of six inches. The seeding was repeated throughout the season at 15-day intervals in order to provide plant material of different ages for experimentation. At the time of emergence, and at various later dates, some seedlings were cut off at ground level and others at one inch below the surface. All the top portions were planted, while the seed parts, in this case, were discarded. The experiment was repeated many times during the summer with similar results. In Table V a typical sample of the results is given to illustrate the extent of regrowth when the plants were sectioned on the 1st, 2nd, 3rd, 4th, 5th, 12th, 13th, 21st, 25th and 32nd day after emergence. The data from this experiment show that, in the field as well as in the greenhouse, seedlings as old as 32 days from emergence can reproduce vegetatively from the top sections cut off a little below the soil surface.

6. Regrowth from One-inch Sections of the Underground Stems of Wild Oats Seedlings Cut at Various Stages of Plant Development

In experiment 5 we were dealing with the top portions of the seedlings cut at different stages of plant development. In this experiment plant material from the same plots was used, but each plant was severed at the soil level and the underground portions were cut into sections one inch in length, beginning at the top. This made six sections for each plant. When these were replanted it was found that practically none of them would grow except the uppermost, that is, the sections in the first inch below the surface. The results indicate that regrowth from short underground sections of wild oats stems can occur under field conditions, and that the nodal tissue is found mainly at this particular depth. The results are shown in Table VI.

7. Regrowth from the Stem above Ground, when the Latter is not Detached from the Original Root System and is Covered with Soil at Various Stages of Plant Development

In experiment 4 the behavior of the top portions of seedlings sectioned at different stages of development and grown under greenhouse conditions was observed. In this present experiment a similar test was made in the field, but

TABLE VI

NUMBER OF NEW PLANTS PRODUCED FROM THE UNDERGROUND SECTIONS OF WILD OATS
SEEDLINGS CUT AT DIFFERENT STAGES OF PLANT DEVELOPMENT,
10 PLANTS BEING USED IN EACH CASE

Depth, in.	Days from emergence when sectioned											
	1	1	1	2	3	4	5	12	13	21	25	32
	Number of new plants produced											
0-1	9	10	8	7	3	8	2	7	6	6	7	9
1-2	0	1	0	0	0	0	0	0	0	1	0	0
2-3	0	0	0	0	0	0	0	0	0	0	0	0
3-4	0	0	0	0	0	0	0	0	0	0	0	0
4-5	0	0	0	0	0	0	0	0	0	0	0	0
5-6	0	0	0	0	0	0	0	0	0	0	0	0

in a slightly different way. Several rows of wild oats were covered with soil at various stages of plant development until only the leaf tips were exposed to light. In the fall, a random sample of 20 plants was taken from each row. Each plant was examined with respect to the number of new root systems and corresponding new plants produced. In this experiment also it was evident that regrowth occurred only at the nodes. The results are presented in Table VII and specimens of rooted stems are shown in Fig. 7.

TABLE VII

REROOTING FROM ABOVE-GROUND NODES OF WILD OATS WHEN THE TOPS WERE COVERED
WITH SOIL AT DIFFERENT STAGES OF PLANT DEVELOPMENT.
TWENTY PLANTS EXAMINED IN EACH CASE

Stage of growth	No. of plants producing new root systems and stems at				Per cent of rerooted plants
	1st node above ground	2nd node above ground	3rd node above ground	4th node above ground	
1. 2nd leaf	1	2	3	9	75
2. 3rd leaf	1	1	3	11	55
3. 4th leaf	2	2	5	17	85

8. Tillering from Nodes of Wild Oats Located Deeply Underground, when the Top Growth was Inhibited

In experiments 4, 5 and 7, it was possible to demonstrate clearly that if sections of the above-ground stem are subjected to favorable conditions for regrowth, the latter invariably takes place from nodes. In experiments 1, 2, 3 and 6, it was found that regrowth can occur from sections of the underground stem as well, yet it was not possible to demonstrate satisfactorily in the latter case whether or not it originated only from nodal tissue. On this account, an attempt was made to force the underground stem of wild oats into active growth by inhibiting the top growth, in which case it might be possible to observe where new roots or shoots occurred.

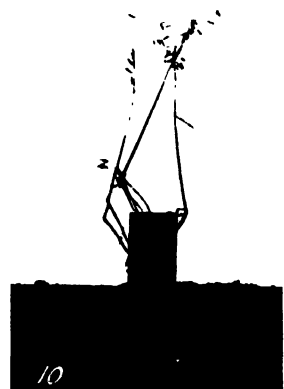
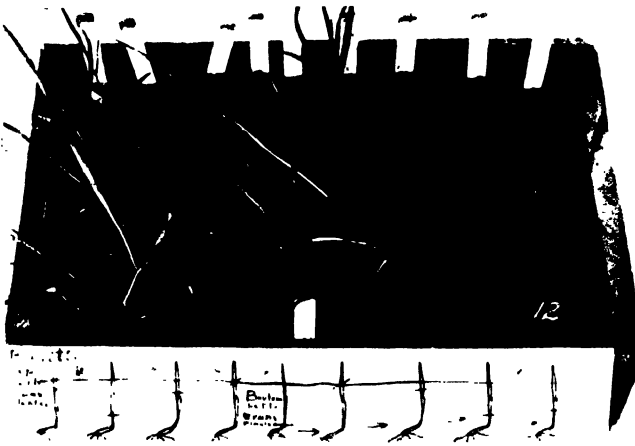
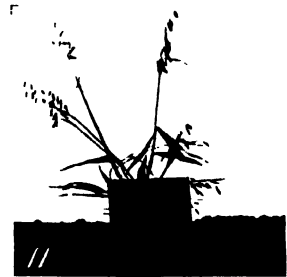
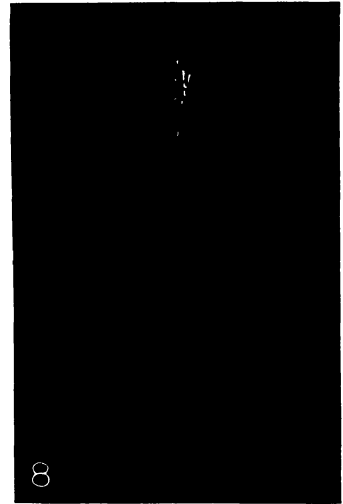
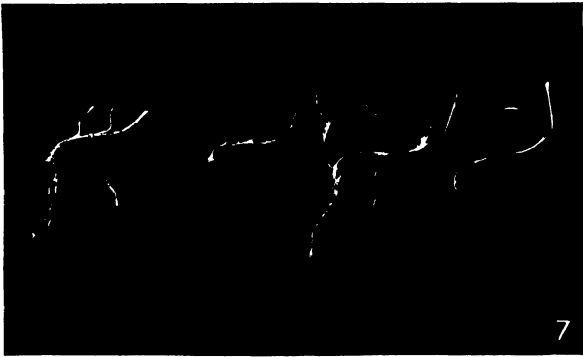


FIG. 7. *Rerooting and tillering at the nodes above ground from stems of plants that had been covered with soil. From left to right: wild oats, cultivated oats, barley, wheat.* FIG. 8. *Underground portion of wild oats plant, grown from a depth of 10½ in. showing buds and secondary roots, developed from nodes at 1, 1½ and 5 in. in depth.* FIG. 9. *Wild oats plants vegetatively produced from the top and underground sections of the stems.* From left to right: 1, tops cut 1 in. under the surface (survival 100%); 2, tops cut at ground level (survival nil); 3, one-inch sections taken at 0 to 1 in. in depth (survival 90%); 4, one-inch sections taken at 1 to 2 in. in depth (survival nil). FIG. 10. *A wild oats plant supported by a single primary root from the two-leaf stage until maturity.* FIG. 11. *A wild oats plant supported by a single secondary root from the seedling stage until maturity.* FIG. 12. *Top portions (left) and seed portions (right) of Banner oats showing the plants which became rerooted and survived. Cultivated oats and wild oats behaved in a similar manner.*

When fully developed, but before maturity, the top growth was cut at soil level. The new shoots which came up soon after were cut again and again. On the 20th day after the first cutting a number of these plants were excavated, carefully washed, and individually examined. Fig. 8 shows a typical specimen which had produced a new root system and tillers from a node 1 in. below the surface. These were purposely cut off before taking the picture, in order to show more clearly the portion of the underground stem at lower levels. At points located at depths $1\frac{1}{2}$ and 5 in., two additional root systems were developed. Examination of untreated plants indicated that these buds would have remained dormant had they not been forced into growth. Not only were new root systems developed, but shoots also appeared at points where the roots emerged. This is additional evidence that rerooting occurs only from nodes.

Fig. 9 shows the luxuriant growth produced from cut sections of wild oats seedlings, and serves to emphasize how important the phenomenon of vegetative propagation may be under certain conditions, in the practical control of this weed in the field. Row No. 1 at the left originated from the top section of seedlings cut 1 in. below the surface. Row No. 2, which produced no plants, was planted from top sections cut at ground level. Row No. 3 grew from one-inch sections taken between the soil surface and 1 in. in depth. Row No. 4, planted with sections between 1 and 2 in. in depth, did not produce a single plant in this case.

9. Regrowth of Wild Oats Seedlings in a Cultivated Field

Information obtained from the eight preceding experiments explains satisfactorily the nature of vegetative propagation of wild oats. From a practical point of view, however, the farmer is interested primarily in whether wild oats seedlings that have been plowed down are likely to grow again. An attempt was made, therefore, to do some work on ordinary farm land infested with wild oats. The conditions in the spring of 1931 proved most unfavorable, but later in the season, field conditions were simulated in the Weed Nursery by seeding wild oats thickly on a piece of land and plowing down the seedlings. This was done at the second-leaf stage (15 days after emergence, on July 12) when temperatures were high and the soil dry.

The soil was plowed 4 in. deep and the furrow left rough until fall. Most of the plants were covered but some of the leaf tips were left exposed, and in other cases the roots were exposed. Because of the dry condition a piece of the field, 100 sq. ft. in area, was watered. Five days later a light rain fell, which penetrated the soil for $\frac{3}{4}$ in., and afterwards the weather was variable with some light precipitation. The plowed block was inspected from time to time until August 20, when final records were taken, both on the watered and unwatered areas. The proportion of plants that survived was 25% in the watered plot and 0.5% in the area which had not been watered. Most of the plants having their leaf tips exposed on the watered plot survived until maturity, and produced seed. On the unwatered area most of the plants were killed. Very interesting, however, were certain plants that were entirely uprooted except

for a single primary or secondary root. These plants were left on the field in this condition until fall without any additional treatment and in all cases they survived, produced several tillers, and matured seed. Two of these are shown in Figs. 10 and 11.

This last experiment gave definite evidence that, even under unfavorable conditions of moisture and temperature, plants which have been injured by farm implements and covered with soil, or which have had nearly all their roots destroyed, can re-establish themselves and grow again. Since only 0.5% of the original number of plants on the unwatered area recovered after being plowed down, while on the watered plot 25% of these survived, it would seem that the number of plants which is likely to become rerooted under field conditions will be directly related to soil conditions which favor or retard vegetative regrowth of the cut sections. It is logical, therefore, to presume that regrowth in the field would occur most frequently following early spring cultivation, when growing conditions are most favorable, and when the work is done in such a manner as to leave the wild oats seedlings partly covered, or otherwise favorably situated for becoming rerooted.

B. Vegetative Propagation in Cereal Crops *Aveneae* and *Hordeae*

Having investigated the phenomenon of regrowth in wild oats, the authors were interested in learning whether the cereal crop plants would behave in the same manner. Some experiments were planned, therefore, with wheat, cultivated oats, barley and spring rye, in order to determine the extent to which these could be propagated from vegetative cuttings.

10. Vegetative Propagation from the Upper and Lower Portions of Wild Oats and Cereal Crop Plants

Seeds of wild oats, cultivated oats, wheat, spring rye and barley were sown in greenhouse flats at a depth of $1\frac{1}{2}$ in., five flats being seeded to each crop. In one set of five flats the plants of each crop were cut in two just above the seed (series No. 1). In series Nos. 2, 3 and 4 the plants were cut $\frac{1}{4}$, $\frac{1}{2}$ and 1 in. above the seed respectively. Those in series No. 5 were cut off at ground level. All plants were sectioned on the fifth day after emergence, and both the top and the lower portion of each seedling were replanted. The results from this experiment are given in Table VIII. Re-established plants are shown in Figs. 12, 13, 14 and 15.

From the results shown in Table VIII, and as illustrated in Figs. 12, 13, 14 and 15, it is evident that there was a striking difference between wild and cultivated oats on one hand, and wheat, rye and barley on the other. In both species of oats it was the top portion which grew, whereas with the other cereals it was the seed portion which produced plants. This indicates a fundamental difference in the morphology of the two tribes *Aveneae* and *Hordeae*. In several special experiments this question was fully investigated and explained as follows: The region of primary elongation of the underground stem in oats is the mesocotyl, which is located between the base of the coleoptile and the seminal roots. In wheat, rye and barley, on the other hand,

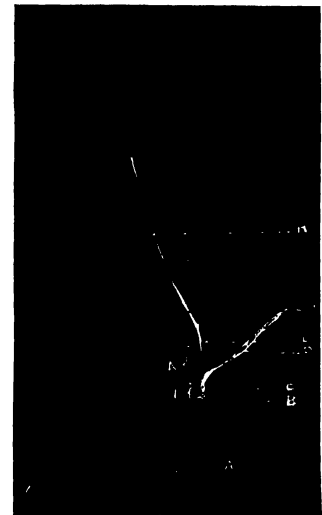
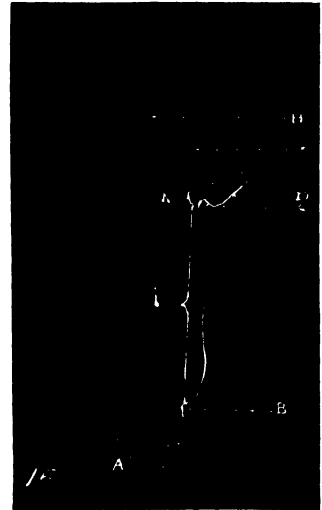
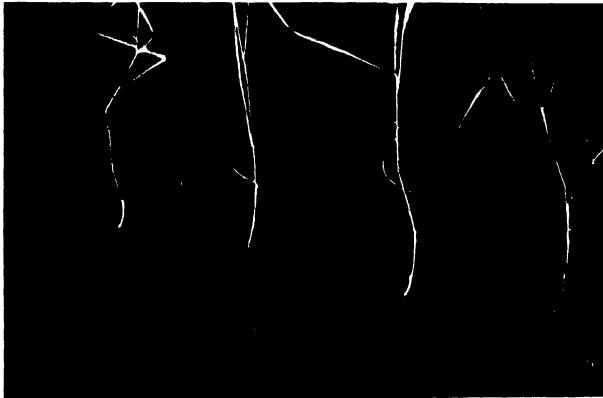
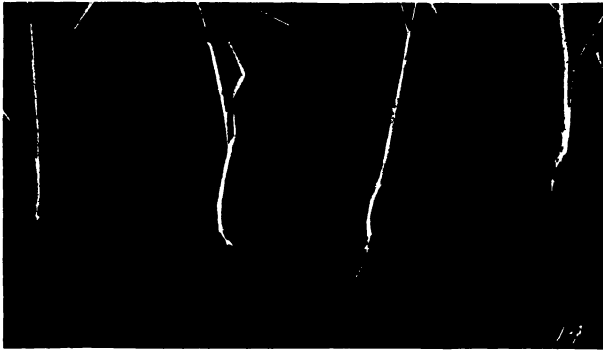
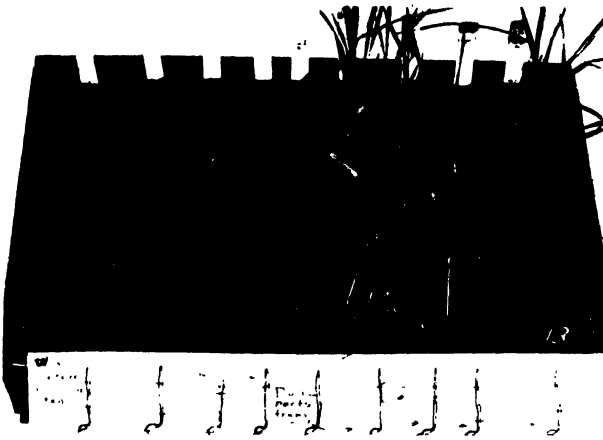


FIG. 13. Top portions (left) and seed portions (right) of Marquis wheat showing the plants which became rerooted and survived. Wheat, rye, and barley behaved in a similar manner. FIG. 14. Plants produced from the top portions of cultivated oats, cut at different levels above the seed (from left to right): 1, $\frac{1}{4}$ in. above the seed; 2, $\frac{1}{2}$ in. above the seed; 3, 1 in. above the seed; 4, at soil surface, the top portion in this case died, but the corresponding lower portion grew. FIG. 15. Plants produced from the seed portions of wheat cut at different levels below the surface. (from left to right): 1, cut at ground level; 2, cut 1 in. above the seed; 3, cut $\frac{1}{2}$ in. above the seed; 4, cut $\frac{1}{4}$ in. above the seed. FIG. 16. Wild oats seedling two days after emergence. FIG. 17. Wheat seedling two days after emergence. Explanatory notes for Figs. 16 and 17: A, seminal roots; B, cotyledonary node; C, coleoptile node; D, coleoptile; E, second node on stem above the seed; F, second green leaf above coleoptile; G, first green leaf above coleoptile; H, mesocotyl; I, first true internode.

TABLE VIII

NUMBER OF NEW PLANTS PRODUCED VEGETATIVELY FROM THE TOP AND SEED PORTIONS OF WILD OATS, BANNER OATS, MARQUIS WHEAT, PROLIFIC SPRING RYE, AND HANNCHEN BARLEY. TEN PLANTS USED IN EACH CASE

Plant	Sectioned	No. of cuttings survived		Per cent of cuttings survived	
		Top part	Seed part	Top part	Seed part
SERIES I					
Wild oats	Close to seed	10	0	100	0
Banner oats		10	0	100	0
Marquis wheat		0	0	0	0
Prolific rye		0	1	0	10
Hannchen barley		0	0	0	0
SERIES II					
Wild oats	One-quarter inch above seed	10	0	100	0
Banner oats		5	0	50	0
Marquis wheat		0	4	0	40
Prolific rye		0	4	0	40
Hannchen barley		0	2	0	20
SERIES III					
Wild oats	One-half inch above seed	9	0	90	0
Banner oats		7	0	70	0
Marquis wheat		0	8	0	80
Prolific rye		0	3	0	30
Hannchen barley		0	6	0	60
SERIES IV					
Wild oats	One inch	7	0	70	0
Banner oats		6	0	60	0
Marquis wheat		0	7	0	70
Prolific rye		0	6	0	60
Hannchen barley		0	8	0	80
SERIES V					
Wild oats	At soil level	1	6	10	60
Banner oats		0	6	0	60
Marquis wheat		0	10	0	100
Prolific rye		0	6	0	60
Hannchen barley		0	10	0	100

elongation takes place in the first true internode, which is the region immediately above the coleoptile node. Figs. 16 and 17 show the fundamental difference between wild oats and wheat seedlings grown from a depth of 3 in. and excavated on the second day after emergence (see also Simmonds (2, 3)).

In the early stages of seedling development, it is the coleoptile node which produces regrowth most readily in both oats and the other cereals. In oats, however, this node is pushed up to the surface by elongation of the mesocotyl, and this is the reason why the seed portion of oats seedlings never produces new plants (see Figs. 12 and 16). In wheat, rye and barley, the coleoptile node remains close to the seed and the region of elongation above it does not become differentiated into distinct nodal tissue until the seedling is somewhat advanced in development. This explains why regrowth does not occur from the tops in these plants when they are cut a little distance above the seed (see Figs. 13 and 17). The seed portions, however, bearing the coleoptile node, are

capable of regrowth (see Fig. 13 and Table VIII). In Fig. 14 four specimens of cultivated oats are shown, three of which were developed from the top portions. In the fourth case the seedling was cut off at ground level and died, but the seed portion survived and produced a new plant. Fig. 15 shows specimens of wheat which developed from the seed portions. The top portions invariably died. This striking contrast in the behavior of the two tribes is directly associated with a difference in their morphology as explained above.

11. Vegetative Propagation from the Top and Seed Portions of Cereal Plants Sectioned at Various Stages of Development

In this experiment an attempt was made to section the plants at later stages of growth. Seedlings of cultivated oats, wheat, rye and barley were cut below the first, second and third nodes above the surface, at ground level, and at 1 in. below the surface. The plants were severed at the one-, two- and three-leaf stages. In all cases the top and seed portions were replanted.

As in the preceding test, the majority of the seed portions of very young wheat, rye and barley plants grew again, while the corresponding top portions died. Ten days after emergence the new growth from the seed portions died also. In the case of older plants, on the other hand, the top portions in many instances produced normal plants. This was especially true of the top portions which included 1 in. of stem below the surface, or the first node above ground.

From the information obtained in this experiment it is obvious that both oats and the other cereals can reproduce vegetatively, but only from the top portions, and in the later stages of development. In the case of wheat, rye and barley, this is due undoubtedly to the fact that at this stage the coleoptile nodes have passed beyond the stage of greatest cell activity. On the other hand, nodes located immediately below and above the soil surface are in a better growing condition, and hence are capable of regrowth.

With regard to the occurrence and vigor of vegetative regrowth in oats and other cereals, it is essential to point out that the former are more capable of regrowth, and the plants thus produced are always more vigorous than those of wheat, rye, or barley.

Field Experiments

12. Regrowth of Cereal Crops Under Field Conditions

Wheat, oats, barley and rye were tested for vegetative regrowth under field conditions in 1931. These were sown at a depth of 3 in. in 8-ft. rows, at intervals throughout the season, on the same dates as were used for wild oats in Experiments Nos. 5 and 6. At emergence, and at various other times, these plants were cut off both at the surface and at 1 in. below the ground. Both the top and seed portions were replanted with the results shown in Table IX.

The plant material in this experiment was sectioned on the 1st, 3rd, 9th, 12th, 24th and 32nd days after emergence. As in all previous experiments, plants of the two different tribes behaved entirely unlike in the early seedling stage, but in a similar manner from approximately 10 days after emergence.

This is exceptionally well shown in Table IX. In the case of seedlings younger than 10 days after emergence, neither oats nor the other cereals produced new plants from the tops when they were cut at the surface. Apparently no nodal tissue capable of regrowth was included in these portions. Oats plants which

TABLE IX
REGROWTH OF CEREAL PLANTS UNDER FIELD CONDITIONS, 1931.
TWENTY SEEDLINGS USED IN EACH CASE*

Plant	Age of seedlings from emergence, days	Number of new plants			
		Cut at one inch below surface		Cut at surface	
		Top portion	Seed portion	Top portion	Seed portion
Wheat	1	0	4	0	6
Wheat	1	0	10	0	10
Rye	1	0	0	0	4
Rye	1	0	2	0	2
Barley	1	0	0	0	4
Barley	1	0	4	0	4
Cultivated oats	1	10	0	0	5
Cultivated oats	1	10	0	0	8
Wheat	3	0	4	0	8
Rye	3	2	0	0	0
Barley	3	0	0	0	7
Cultivated oats	3	10	0	0	4
Wheat	9	0	0	0	0
Rye	9	2	0	0	0
Barley	9	0	0	1	1
Cultivated oats	9	—	—	—	—†
Wheat	12	10	0	0	0
Rye	12	9	0	2	0
Barley	12	10	0	0	0
Cultivated oats	12	10	0	0	4
Wheat	24	0	0	0	0
Rye	24	8	0	0	0
Barley	24	—	—	—	—†
Cultivated oats	24	10	0	0	0
Wheat	32	10	0	0	0
Rye	32	9	0	0	0
Barley	32	9	0	0	0
Cultivated oats	32	—	—	—	—†

*The results from 10 plants are given in columns 1 and 2, and from the other 10 plants in columns 3 and 4.

†Plants were damaged.

were cut at 1 in. below the surface grew again in all cases, but none of the other cereals did so. This was due to the fact that the nodal tissue in oats seedlings was pushed up to the surface by the elongation of the mesocotyl, and thus was included in the tops. In the other cereals the first (coleoptile) node remained close to the seed, while other nodes which might develop later near the surface had not, at this time, been differentiated sufficiently to initiate

new growth. Hence, the top portions of wheat, rye and barley at this early stage, even when cut at 1 in. below the soil surface, contained no nodal tissue capable of regrowth, and all of them died. Somewhat similar results are shown in Table IX in the case of cuttings taken from seedlings three and nine days after emergence. Where material was sectioned at later stages of development on the 12th, 24th and 32nd days after emergence, the results for cereals resemble those for oats sectioned at very early stages.

The vigor of vegetative regrowth in different species is of interest. With both species of oats, rerooting takes place with great readiness and the plants thus produced are as vigorous as those produced from seed, while with other cereals, when regrowth does occur, it is much less vigorous, and has less resistance to unfavorable external conditions.

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SPIROCHAETES OF THE DISTRICT OF MONTREAL AND OF THE PROVINCE OF QUEBEC¹

BY G. GARDNER

Abstract

This paper is devoted to a brief discussion of the general characteristics of spirochaetes, including staining and cultures, and to records of observations on spirochaetes in Canada, which are of interest for comparison with those from other parts of the world.

Fresh water from various sources was examined both directly and after collection and cultivation. The results show that while Canadian waters are extremely poor in spirochaetes they do contain various types resembling those recorded from other parts of the world. By maceration of vegetation in sterile tap water a number of spirochaetes were obtained including *S. biflexa*, *S. pseudo-icterohemorragiae*, and in particular a distinct type referred to as the "timothy type", obtained from timothy grass. This spirochaete resembles *S. plicatilis*, but is very much shorter (a maximum of 15 μ as compared with lengths up to 500 μ). The addition of oxazine (1-1000) causes the surface membrane and granulations to become distinct and also reveals the existence of an axial filament.

The intestines of a number of animals, both vertebrate and invertebrate, were examined with negative results, but spirochaetes were obtained by cultivation from the saliva or foam of a cow. A number of rabbits were found to be infected with *S. cuniculi* and to harbor a spirochaete in the caecum. *S. icterohemorragiae* was also detected in the kidneys of wild rats.

Strains of *S. pseudo-icterohemorragiae* (free living) were found to be non-pathogenic. Nevertheless after being cultivated for two or three years, some of these free living strains acquire certain characteristics of the pathogenic type and it is possible that under proper conditions the physiology of certain micro-organisms could be so changed as to render them pathogenic to man.

The influence of cold on spirochaetes was also studied, cultures of *S. pseudo-icterohemorragiae* being kept at a temperature of approximately -15°C . The spirochaetes were found to lose their motility gradually and to break up into granules. Subcultures of these forms were negative. It would seem that the comparative rarity of spirochaetes in Canada may be attributed to the low winter temperatures.

Introduction

The word spirochaete was first introduced into the literature by Ehrenberg (10) in 1838, who at that time was studying two new groups of organisms, Spirillum and Spirochaeta. Owing to a misunderstanding that is difficult to explain, the word and the description of Spirillum have been applied to Spirochaeta, and *vice versa*. Thus, spirochaetes are described as spiral organisms, whereas in reality, they are helicoidal or screw-shaped. Pettit (22) was the first to call attention to this error, the seriousness of which may be judged from the following descriptions of a spirillum and a spirochaete.

Definition of a spirochaete. A unicellular protist, related to the Schizophytes, body flexible, forming a perfect geometric screw, no flagella visible under dark field illumination, movements resembling those of a screw.

Definition of a spirillum. A bacterium, body non-flexible, flagella visible under dark field illumination.

¹ Manuscript received December 17, 1931.

Contribution from the University of Montreal, Montreal, Canada. This paper was constructed from a thesis presented to the University of Paris in partial fulfilment of the requirements for the degree of Doctor in Natural Sciences.

Spirochaetes

Spirochaetes possess few morphological or physiological characteristics useful for the determination of species. The few useful characteristics are as follows:

Shape. The shape is of morphological significance in that the dimensions of the helix are to a certain extent characteristic for each species.

Helical amplitude. The helical amplitude of the screw is usually difficult to measure as it is of the order of $0.25\ \mu$ to $1\ \mu$, and in very minute species such as *Sp. biflexa* it may even be impossible to measure it. The helical amplitude varies with different culture media, etc.

Length. The length as well as the helical amplitude varies with different individuals, culture media, etc., and for this reason cannot always be relied upon as an index for the determination of species; for instance, the length of *Sp. eurygyrata* varies between $4\ \mu$ and $80\ \mu$.

Study in vivo. Spirochaeta are best studied *in vivo* under dark field illumination. If the micro-organisms are too active it may be necessary to wait a few minutes to allow the field to clear, but by this means it is always possible to make a distinction between spirochaetes and any other micro-organisms which may appear on the slides. The only objection to the method may be the relative scarcity of spirochaetes, but this difficulty may be overcome by enriching the cultures, thus increasing the relative numbers of spirochaetes.

Experimental

Materials and Methods

The author was obliged to devise special culture methods, since spirochaetes, being neither bacteria nor protozoa, will not develop in the culture media commonly used for such organisms. The technique finally adopted was a modified form of that devised by Dr. M. Zuelzer; spirochaetes being found under dark field illumination, the liquid containing them is centrifuged and the bottom portion transferred to a culture medium.

Some of the principal media used were ordinary hay infusion, 1% peptone in boiled tap water, and solidified agar. The further details of procedure and media will be discussed in connection with each particular case.

Staining

With the exception of a study *in vivo* the best results were obtained by staining with basic aniline dyes, and of these, crystal violet and Ziehl's fuchsin or phenol fuchsin gave the best results. The staining techniques were as follows:

Pulgher technique. Smears are prepared, allowed to stand for two hours and then washed with distilled water. A small quantity of the stain (as per formula) is boiled in a test tube, poured on the slide, allowed to cool, and washed with tap water. Formula of Pulgher stain:— distilled water, 100 cc.; alcoholic tannin, 0.90 cc.; crystal violet, 0.45 gm. After cooling this is mixed in equal parts with a 3% solution of hydrochloric acid in distilled water.

Renaux technique. Smears are fixed with Ruge's formalin (2% formalin in 1% acetic acid) for from two to four minutes. They are then washed with alcohol and covered with a saturated aqueous solution of picric acid. After

standing 10 min. they are washed and stained with Ziehl's fuchsin, made up as follows: fuchsin, 1 gm.; carbolic acid, 5 gm.; absolute alcohol, 10 cc.; distilled water, 100 cc.

The fuchsin is ground with alcohol in a mortar, adding the carbolic acid meanwhile. Two-thirds of the distilled water is then slowly added and the mortar is rinsed with the remaining one-third. The solution is allowed to stand 24 hr., filtered and kept in a well corked bottle.

Silver nitrate method. A silver nitrate method similar to that used in staining nerve tissue is frequently used in the study of spirochaetes, but while the results are striking in appearance the method has the disadvantage that the interior of the helix is completely filled with silver so that it is possible to mistake connective tissue fibres for spirochaetes. On account of the possibility of such an error, the method was not used to any very great extent in the present studies.

Discussion

The spirochaetes studied were those present in water, cow manure, plant infusions, algae and animals. They will be discussed in that order.

Spirochaetes of Water

Spirochaetes have been found in soft waters in many different countries, especially in the following: in Germany by Uhlenhuth (24), Baermann (1), Zuelzer (31, 32) and Hoffmann (15, p. 611); in England by Dobell (9) and Hindle (14); in France by Pettit (22), Etchegoin (11, 12), Cantacuzene (5) and Vincent (28); and in the United States by Wolbach and Binger (29, 30), and by Noguchi (18, 19, 20, 21).

In Canada the study of Spirochaetes has not attracted much attention in the past though *Treponema pallida* is well known on account of its medical significance, while *Spirochaeta icterohemorrhagiae* has been observed in Toronto, by Bates (2) and by Cameron and Irwin (4).

In the present study a large number of samples were examined including some from rivers condemned by the Board of Health, but spirochaetes were observed in only a few cases. The spirochaetes found in rivers resembled more or less those found under similar circumstances in Europe, though in many cases considerable differences were observed.

Spirochaetes were present in samples of water from the following places: Campbellton Wharf (sea water), Cross Point (dirty water from a gutter), Matapedia River at Matapedia, Quebec, St. Lawrence River water at Tadousac in Quebec (this water had been lying, for some time, in holes in the wharf), in water from Rivière du Loup at the city of Rivière du Loup in Quebec, and in some water from a tannery. A detailed discussion of these samples follows.

Sea water from Campbellton, N.B. This water, in which there was a small quantity of sand, contained two types of Spirochaetes. These may be described as follows:— Type No. 1: length 5-9 μ , total width 1-1.5 μ , helix with 2-6 loose turns, membranes and granulations in the body visible. Type No. 2: length 10-15 μ , width 0.5 μ , helix with 20-30 very close turns, extremities fine, resistant to 10% saponin, division transversal.

Type No. 1 was similar to *Sp. plicatilis pallida* Zuelzer, but was somewhat thicker and shorter, while Type No. 2 was similar to *Sp. stenostrepa*. A complete study was impossible owing to a scarcity of specimens.

Cross Point. This water contained spirochaetes which may be described as follows: length short, helix with 2-4 turns, measuring 2-3 μ , membrane granular.

Matapedia River. Large spirochaetes showing granulations were found.

St. Lawrence River at Tadousac. This water contained large spirochaetes, length 4-5 μ , membrane granular, similar in appearance to *Sp. plicatilis marina* except for the great difference in length, the latter measuring 125 μ in length. The spirochaete found in the Campbellton water and resembling *Sp. plicatilis pallida* was also present.

Rivière du Loup. Spirochaetes 3-5 μ in length and with transverse division were found.

Tannery water. Samples of water and other liquids used for tanning leather were examined, two types of spirochaetes being observed: Type No. 1: length 2-10 μ , helix with 4-8 turns, membrane visible, granulations absent, individuals instantly dissolved by 30% Javel water. Type No. 2: length 2-4 μ , and 6-9 μ , total width 1 μ , granulations visible. Type No. 2 showed a strong resemblance to *Sp. pseudo buccalis* observed by Zuelzer in Germany.

No spirochaetes were found in tap water in the city of Montreal or in water from two taps in Dr. Pettit's laboratory at the Pasteur Institute in Paris.

Spirochaetes in Cow Manure

From time to time spirochaetes with from two to three turns have been observed in cow manure. In the present study the manure was diluted with boiled water and cultured on agar. A few spirochaetes were obtained in this way but not a sufficient number for a complete study of their characteristics.

No spirochaetes were found in liquid manure though good specimens of *Spirillum* 15 μ in length, 1-2 μ in width and with from three to four undulations were found. These were present in numbers as high as 15-25 in each field.

Spirochaetes of Plant Infusions

A great number of plants were examined in the hope of finding spirochaetes, the best results being obtained with infusions of timothy, *Phleum pratense*. A number of other plants, including *Euphorbia*, potatoes and beans, were examined in an unsuccessful attempt to find spirochaetes. Fresh plants were collected and brought directly to the laboratory where they were placed in sterilized jars containing boiled tap water.

Spirochaetes were usually present after three weeks, and may be described as follows: average length 6-15 μ , width 0.25-0.50 μ , helix with 2-8 turns (pH; 6.6-6.8). These spirochaetes represent a new species which will be discussed later. They were found in some infusions for over three months and may be temporarily referred to as *Spirochaeta of timothy*.

Other micro-organisms recognized in these infusions were: *Bodos* (sp?) which appear a few days before the spirochaetes and remain two or three weeks longer; *Sp. biflexa*; and a large bacterium with long flagella which, although observed by Noguchi (21), has not yet been named.

It is of interest to compare the morphological characteristics of *Sp. of timothy* with those of various other common spirochaetes. This comparison is made in Table I.

TABLE I

A COMPARISON OF THE MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF *Sp. of timothy* AND THOSE OF VARIOUS OTHER SPIROCHAETES

Name	Length in microns	Width in microns	Stability of shape with various media	Stability of shape when stained	Remarks
<i>Sp. of timothy</i>	6-15	.25-.50	Retains shape	More change than in case of <i>pseudopallida</i> or <i>recurrentis</i>	Similar in appearance to <i>Sp. plicatilis</i> , but shorter, aerobic, retained by Chamberland L3 filter
<i>Sp. graminea</i>	100	2.5	Shape irregular	Shape irregular	Similar in appearance to <i>Sp. stenostrepa</i> , less refringent
<i>Sp. plicatilis</i>	500	.25-.50	Changes shape slightly	Axial filament strongly stained	See remarks on <i>Sp. of timothy</i>
<i>Sp. recurrentis</i>	6-15	Greater than <i>Sp. timothy</i>	Changes shape	Less change than in case of <i>Sp. of timothy</i>	
<i>Sp. calligyrum</i>	Shorter than <i>Sp. timothy</i>	Thinner than <i>Sp. timothy</i>			Anaerobic, screw heavier than <i>Sp. pallida</i>
<i>Sp. biflexa</i>	5-7	0.2	Retains shape	Slight changes, ends remain curved	Number of turns in helix similar to <i>Sp. of timothy</i>

From Table I it is apparent that *Sp. of timothy* is not identical with any of the other spirochaetes in the table, and in view of other careful comparisons yielding similar results the author believes it to be a new species.

It appears to be fairly common, since it can be obtained from either green or dried timothy, or even from material that has been in the field during the winter. Growth can be stimulated by the addition of 1% peptone to the infusions. It is also possible to obtain it from hay infusions kept at pH 6.6 for a month. An unsuccessful attempt was made to secure pure cultures in Montreal and at the Pasteur Institute in Paris.

A few additional notes on the morphology and physiology of *Sp. of timothy* follow.

Morphology. *Sp. of timothy* sometimes contains granulations, and after staining, the body flattens slightly, though the screw remains apparent. The membrane covering the contours is clearly visible and represents about one-fifth of the total thickness of the organism.

Physiology. *Sp. of timothy* shows interesting reactions to various chemical substances. It is killed after a few minutes contact with bile salts (10%), and is dissolved after half an hour's contact with the same solution. It is rapidly dissolved by commercial Javel water diluted to one-third with ordinary water. Saponin (10%) produces granulations in the body.

A few experiments were also made with oxazine. On placing the organisms in an aqueous solution of oxazine (1/1000) they were not stained but the membrane and granulations became more distinct while the axial filament in particular became very distinct. After some time death occurred, the remaining portions, *i.e.*, the axial filaments, adhering to each other in chains, forming figures-of-eight.

The organism normally moves with a screw-like motion but on reaching the granular stage the movement becomes slower and finally stops.

It was thought at the beginning of the present study that the pH would vary considerably in the cultures, but the contrary proved to be the case, and after numerous observations it was discovered that in the case of any one culture, a decrease in the number of spirochaetes was due to an exhaustion of organic material rather than to a change in pH.

Bodos (sp?) were sometimes very numerous in the author's cultures, and proved to be a sure indication of the future presence of spirochaetes. There is possibly a symbiotic relationship between the two.

Spirochaeta biflexa

It has been mentioned that *Sp. biflexa* sometimes occurred in the author's cultures, particularly in infusions of timothy. This was however only an accidental host.

Sp. biflexa has been found in the water of Jamaica Pond, in Boston, by Wolbach and Binger (30) and has been studied in Germany by Zuelzer (31, 32) and in America by Dimitroff (6, 7, 8) and by Wolbach and Binger (29, 30).

The correct name of this spirochaete has long been a controversial matter; we shall therefore use the name first applied to it. Zuelzer considered that the species *Sp. pseudo-icterogenes* discovered by her was identical with *Sp. biflexa*, but this identity is doubtful since the former is sometimes pathogenic for the guinea pig, the latter never.

Sp. biflexa seems to be world-wide in distribution though individuals are always scarce.

The organism as found in the author's cultures differed slightly from that found by Wolbach and Binger as is shown by Table II.

Sp. pseudo-icterohemorrhagiae

This organism was found in some of the author's cultures but individuals were never numerous. They appeared to be very similar to the European species, their description being as follows: length 10-15 μ , helix with 16-22 turns, active individuals with very delicate coils.

The position and importance of this organism in nature will be discussed later.

TABLE II
A COMPARISON OF THE MORPHOLOGICAL CHARACTERISTICS OF *Sp. biflexa*
AS DETERMINED BY WOLBACH AND BINGER AND BY GARDNER

Authority	Length in microns	Width in microns	Helical amplitude	Number of turns in helix
Wolbach and Binger	5-7	0.2	0.2-0.25	22-32
Gardner	10-12	0.3	0.2	20-40

Spirochaetes in Algal Infusions

Infusions were also made of blue and green algae taken from a spring. Boiled tap water was added to the culture which shortly became filled with bacteria, spirilla and diatoms of various species. Spirochaetes appeared in 10 days. The species present were: *Sp. of timothy*, *Sp. biflexa* and *Sp. plicatilis*.

Spirochaetes of Animals

Considerable work was done on animals in an attempt to discover spirochaetes but it was found that only a small proportion of those examined harbored these organisms.

Cow. The saliva or foam from the mouths of several cows was examined as long as possible after feeding. It was found to be difficult to obtain these materials between periods of rumination. On examination under dark field illumination no spirochaetes were seen, but were obtained in from two to four weeks on placing the saliva in boiled tap water with peptone, or upon hay agar. They were of course mixed with great numbers of bacteria.

These spirochaetes may be described as follows: length 3-10 μ , width about 1 μ , helix with 2-6 well developed turns, membrane visible, body containing granulations. Dr. Noguchi (21), in the United States found a spirochaete in the gastric mucosa of an ox; he states, "I detected a minute *Leptospira* in the gastric mucosa of the ox (*Leptospira bovis* n.sp.)." This description is so brief that it is not possible to make any comparison between the two organisms.

It was thought at first that these spirochaetes might resemble those from timothy, since in the province of Quebec, cows are fed, to some extent, on this plant. Examination showed, however, that the two differed greatly in both length and width.

Rabbit. In the province of Quebec as well as in other countries rabbits are parasitized by a spirochaete, *Sp. cuniculi*, and this phenomenon has been the subject of much study by many investigators. The species was observed by the author on many occasions and found to have the following characteristics: length 1-16 μ , width 0.25 μ , helix with 6-9 or 16 turns.

This species is so much like *Treponema pallida* that many workers are unable to distinguish between the two without inoculation.

A second species of spirochaete, bearing a close resemblance to those found by Sanarelli (23) in the caeca of Italian rabbits was found by the writer in the caeca of Canadian rabbits and appears to have been observed only by Sanarelli and the author.

The method used in this case was as follows: the contents of the caecum were placed in boiled tap water with added peptone and after the appearance of spirochaetes some of the liquid was transferred to a tube with an agar slant, adding enough liquid to cover the slant completely. The organisms usually grew more abundantly in the upper part of the liquid.

The only differences between the spirochaete found by Sanarelli and that found by the author is that the latter is slightly thicker, and that the extremities are blunt instead of pointed. It bears little resemblance to *Sp. eurygyrata* of the human intestine, this species varying in length from 4-56 μ , while that obtained from rabbits was relatively constant in length.

Rat. Spirochaetes very similar in appearance to those found in rabbits were also found in the caecum of a rat. The kidneys of the same rat contained *Sp. icterohemorragiae* (see next paragraph).

The Problem of Sp. icterohemorragiae in Nature

The work of Inada and his coworkers (16) has shown a relationship between human spirochaetosis and the presence of *Sp. icterohemorragiae* in the soil. This observation was the beginning of research in many different parts of the world. Uhlenhuth and Fromme (25) discovered in stagnant water near Berlin a spirochaete having all the characteristics of *Sp. icterohemorragiae* and named it *Sp. icterogenes* Uhlenhuth and Fromme. It was renamed *Sp. pseudo-icterogenes* Uhlenhuth and Zuelzer, and later Dr. Zuelzer believed it to be identical with *Sp. biflexa* Wolbach and Binger. Later, Noguchi (21) searched for *Sp. pseudo-icterogenes* in various regions of the United States and found "organisms indistinguishable from *Leptospira icterohemorragiae*."

Professor Inada has given to the micro-organism of spirochaetal jaundice the name of *Sp. icterohemorragiae* and the author believes with Dr. Pettit that the correct name for free living spirochaetes of the group bearing such a strong resemblance to *Sp. icterohemorragiae* should be *Sp. pseudo-icterohemorragiae*.

Sp. icterohemorragiae may be described as follows: average length 9 μ , extreme lengths 3-40 μ , helix with about 20 turns, helical amplitude about 0.5 μ , division transverse, an ecto-parasite of cells, very pathogenic for the guinea pig, less so for man, the causal agent of a jaundice with karyokinesis, and hematophagia, may be cultivated on relatively non-nutritious media, physiological serum, rabbit serum, etc., optimum temperature 29° to 30° C., optimum pH 7.7, does not resist heat, desiccation, traces of acids, bile salts *in vitro*, and is not attacked by 10% saponin. It is agglutinated and lysed by antispirochaetic serum, and by the serum of patients that have recovered from spirochaetal jaundice.

This description is sufficient to identify a micro-organism living in the tissue of a mammal, but it is another matter to identify a free living spirochaete of the groups *icterohemorragiae* or *pseudo-icterohemorragiae* as all the species are so similar in appearance. Morphological characters are not sufficient to distinguish *Sp. icterohemorragiae* from *Sp. pseudo-icterohemorragiae*, and as for pathogenicity, spirochaetes of neither group are necessarily pathogenic for the guinea pig when taken directly from the soil or from water.

Buchanan (3, p. 48) did succeed in producing spirochaetal jaundice in two guinea pigs by inoculating them with mud, but the case is unique, and even Buchanan himself was not able to repeat the result. The author attempted to do so at the Pasteur Institute, but failed.

It appears then that the pathogenicity of free living *Sp. icterohemorrhagiae* cannot be determined positively. Serological characters are not of much use, the antispirochaetal serum of Pettit and the serum of patients convalescent after spirochaetal jaundice having no effect upon free living *Sp. pseudo-icterohemorrhagiae*.

The author has observed numerous strains of *Sp. pseudo-icterohemorrhagiae*, none of which were pathogenic for the guinea pig, the serological reactions being uncertain. It was then thought that possibly these characteristics are acquired only after several passages, through the bodies of animals or cultures, the hypothesis being based on the following experiment with a *Sp. pseudo-icterohemorrhagiae*, Vinzent's strain.

Vinzent (28) in 1926 isolated a strain of *Sp. pseudo-icterohemorrhagiae* from sewer water and having no time to continue his work with that strain, left it at the Pasteur Institute in Dr. Pettit's laboratory. The object of the author's experiment was not to verify the experiments of Vinzent and Michailoff, a later worker with that strain, but rather to determine if, after a year of passages, certain properties of the micro-organism had not been modified.

Comparative tests before and after the series of passages were conducted with *Sp. icterohemorrhagiae* and *Sp. pseudo-icterohemorrhagiae* in connection with Pfeiffer's phenomenon and agglutination, the results being as follows: In the case of Pfeiffer's phenomenon, the results were about the same for both species but the reaction was more intense in the case of *Sp. icterohemorrhagiae*. As regards agglutination both organisms are in general agglutinated by convalescent's serum but *Sp. pseudo-icterohemorrhagiae* is agglutinated only by sera that strongly agglutinate *Sp. icterohemorrhagiae*.

In 1927 Michailoff (17) wrote: "Vinzent's strain was not agglutinated by anti-icterohemorrhagiae serum at 1/100, 1/50 and even 1/10." The main fact of these experiments is that previous to the series of transfers Vinzent's strain was not agglutinated but after the series it was so affected. The hypothesis may then be proposed that free living forms may have physiological properties quite different from those of individuals of the same species taken from the bodies of animals. It is possible that under certain conditions the physiology of certain micro-organisms could be so changed as to render them pathogenic to man.

Effect of Low Temperatures on Spirochaetes

Infusions and cultures of various spirochaetes (those found in timothy, liquid manure, and in the caeca of rats and rabbits) were exposed to low temperatures and it was observed that the individuals became shorter, division ceased or became less intense, the helices became less delicate, narrower and more compact, and that the production of coccoid bodies was hastened.

The organisms did not seem much affected by exposure to ordinary low temperatures of only a few hours duration but were killed by a prolonged exposure

to a temperature of -2°C . They were not affected by exposure to a temperature as low as -20°C ., provided that the exposure was not greater than 30-60 min.

In these experiments 20 tubes were inoculated with a pure culture of *Sp. pseudo-icterohemorragiae* and kept for four days at laboratory temperature. The cultures developing well, 16 tubes were placed in a refrigerator at a temperature of -15°C ., the other four remaining at laboratory temperature.

After 27 days, the tubes kept in the refrigerator showed only small spots or dots under dark field illumination, while in the case of those kept at laboratory temperature, it was possible to obtain spirochaetes by inoculating other tubes from them. It proved impossible to obtain spirochaetes by inoculation of other tubes with material from the tubes kept in the refrigerator.

The fact that the tubes kept in the refrigerator contained only "coccoid bodies" disproves the hypothesis that these bodies are part of any mechanism of division. They represent rather disintegration products.

Gieszczykiewicz (13), working in Dr. Pettit's laboratory obtained much information with regard to these coccoid bodies. The author's results confirm the findings of Gieszczykiewicz.

Conclusions

An attempt has been made to determine the representative spirochaetes of Canada, a certain number of free living and a few parasitic or commensal species being found. It seems that cold is responsible for the scarcity of spirochaetes in the Montreal district.

Types of spirochaetes very similar to those found in Europe were observed, the small differences being due, perhaps, to the differences in local conditions. Other types were noticed, but owing to the difficulties of nomenclature in spirochaetes, the author prefers not to name them.

The question of the screw shape of spirochaetes was considered, and the presence of the axial filament in living spirochaetes was demonstrated by the use of oxazine.

The presence of *Sp. icterohemorragiae* in Montreal rats and of *Sp. pseudo-icterohemorragiae* in a free living condition was demonstrated. It was also observed that free living strains of the latter have physiological characteristics differing from those of individuals obtained from the bodies of mammals, but that after a long series (2-3 years) of passages through the bodies of mammals or cultures, the physiological characteristics of the free living type approached those of the parasitic type. It was then suggested that certain normally harmless micro-organisms might become pathogenic by reason of serial transfer in the above manner.

Finally the effect of low temperatures on spirochaetes was investigated and a series of observations made on the effect of an exposure to -15°C . This exposure resulted in certain morphological changes culminating in death.

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REVIEWS AND NOTES

LIST OF FUNGI INFECTING MAN IN MANITOBA¹

BY A. M. DAVIDSON² AND P. H. GREGORY³

In the course of investigations in medical mycology now in progress in Winnipeg under a grant from the Banting Research Foundation, Toronto, records have been kept of cases of fungus infection treated at the General Hospital and at the Children's Hospital, Winnipeg. The list of species isolated is published with the belief that it will prove of interest for comparison with similar lists compiled by workers in other localities.

The present account is preliminary in scope. The period covered by the observations extended from September 1930 to June 1932. The nomenclature used by Sabouraud has been adhered to as being that most widely known. *Microsporon felineum* Fox and Blaxall, is provisionally taken as including *M. lanosum* Sabouraud.

The number of patients from whom each species has been isolated is given in the right hand column of Table I. The fungi were determined in culture on maltose "Proof medium", in the preparation of which were used maltose "bruit de Chanot" and peptone "Chassaing" as postulated by Sabouraud. In addition, Dr. E. Muskatblit of New York has kindly identified a number of the cultures.

In recording a somewhat smaller proportion of fungal lesions of the glabrous skin (ringworm of the feet, etc.) than probably occurs, Table I does not present quite a true picture of the incidence of the various clinical types of skin infection in Winnipeg. The cause of this is that up to the present time work has been concentrated largely upon affections of the hairy skin. On this account some cases of epidermophytosis may possibly have escaped investigation in the laboratory.

From the data given in Table I, several interesting facts emerge:

- (a) There is a comparative paucity of species of dermatophytes so far recorded in Manitoba. Sabouraud (3, p. 582) isolated 28 species from 500 cases of dermatomycosis in Paris. Burgess (1) in Montreal isolated 23 species from 99 cases.
- (b) A striking divergence from the records of Sabouraud (3, p. 582) is the relative infrequency in Winnipeg of cases of tinea capitis caused by species of *Trichophyton*. This is emphasized in Table II, in which the data of Burgess for Montreal and of Catanei for Oran, Algeria, are included for comparison.
- (c) *Trichophyton crateriforme* Sabouraud (*T. tonsurans* Malmsten), responsible for 112 out of Sabouraud's 377 cases of tinea capitis, has not been isolated at Winnipeg.

¹ Manuscript received June 13, 1932.

² Contribution from the University of Manitoba, Winnipeg, Manitoba.

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TABLE I

LIST OF FUNGI INFECTING MAN IN MANITOBA 1930-1932

	Origin	Number of isolations
<i>Microsporon audouini</i> Gruby		38
tinea capitis	36	
tinea corporis	2	
<i>Microsporon felineum</i> Fox and Blaxall		18
tinea capitis	12	
tinea corporis	8	
infection from cat suspected	9	
<i>Trichophyton violaceum</i> Sabouraud		4
tinea capitis	4	
<i>Trichophyton (gypseum)</i> spp. Sabouraud		4
tinea barbae	2	
tinea corporis	1	
tinea pedis	1	
<i>Trichophyton (faviforme) album</i> Sabouraud		4
tinea capitis, kerion	1	
tinea corporis	1	
tinea barbae	2	
tinea ciliaris	1	
<i>Epidermophyton inguinale</i> Sabouraud		2
tinea cruris	2	
tinea pedis	1	
<i>Achorion schoenleini</i> (Lebert) Remak.		4
favus	4	
* <i>Penicillium (Scopulariopsis) brevicaulis</i>		1
tinea corporis	1	
† <i>Monilia (Candida)</i> spp. (<i>M. albicans</i> ?)		10
moniliasis of mouth	3	
erosio interdigitalis blastomycetica	8	
Total		85

*Pathogenicity regarded as doubtful.

†Pathogenicity regarded as doubtful in certain cases.

TABLE II

DISTRIBUTION OF SPECIES OF DERMATOPHYTES CAUSING TINEA CAPITIS IN
PARIS, MONTREAL, ALGERIA AND WINNIPEG

Organisms causing tinea capitis	Number of cases			
	Paris 1907-1909	Montreal 1925	Algeria 1931	Winnipeg 1930-1932
<i>Microsporon</i> species	159	41	1	48
<i>Trichophyton</i> species	218	13	28	5

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THE ACTION OF ANILINE ON GLUCOSE IN WATER SOLUTION IN THE PRESENCE OF ACETIC ACID¹

BY C. N. CAMERON² AND G. H. GUEST³

Abstract

It was found that water solutions of glucose, aniline and acetic acid yield a brown amorphous precipitate. Further, this precipitate was produced as a result of the preliminary formation of glucose-anilide and then by the further action of acetic acid on the anilide.

The amount and speed of formation of glucose-anilide (produced in the solution of glucose, aniline and acetic acid) were found to increase to a marked extent with increase in the concentration of aniline and acetic acid.

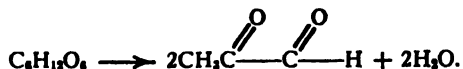
Evidence for the conversion of the stable amylene-oxide glucose-anilide to the more reactive glucose anil was obtained.

Attempts to separate the colored material into different fractions and to identify them are described.

Introduction

When glucose and aniline in alcoholic solution are heated together, a comparatively stable compound, glucose-anilide, is formed. On the other hand, when glucose in alcohol is allowed to react at room temperature with aniline in the presence of acetic acid, or when acetic acid is added to glucose-anilide in alcohol, the reaction apparently goes further and results in the production of brown substances containing nitrogen (3, 4, 5).

The formation of these nitrogenous condensation products may be explained in various ways but all of the explanations involve the supposition that the amylene-oxide glucose-anilide is first changed to glucose anil, $\text{CH}_2\text{OH} \cdot (\text{CHOH})_4 \cdot \text{CH} : \text{NC}_6\text{H}_5$. This may then be hydrolyzed, and the aldehyde-glucose broken down to methyl glyoxal,



The methyl glyoxal may then react with the aniline to give nitrogenous ring compounds. With benzylamine, glucose and acetic acid, it has been

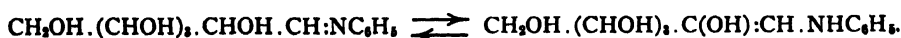
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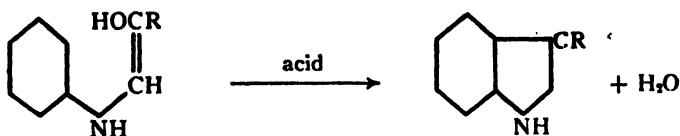
² Late Professor of Chemistry, University of Saskatchewan.

³ Graduate student, University of Saskatchewan.

shown (5) that methyl glyoxal is formed. The glucose anil may change to the corresponding enol form and this by condensation may form the final products, thus:



This enol compound may then condense to an indol derivative, as follows:



The experimental part is a study of the reaction of glucose, aniline and acetic acid in water-solution. It was found that this reaction in aqueous solution is similar to that when alcohol is the solvent (3, 4, 5).

The extent and speed of formation of glucose-anilide, as measured by the polariscope, in solutions of glucose, aniline and acetic acid, increased to a marked degree with increase in the concentration of aniline and acetic acid.

The disappearance of aniline from the reaction mixture was followed by means of Vaubel's reagent (9)†, and of glucose, by means of Bertrand's reagent (1).

Solutions of glucose, aniline and acetic acid showed a reactivity greater than that of any one of the components. Thus, in such solutions of glucose, aniline and acetic acid, two days old, the aniline was more readily oxidized to quinone by potassium dichromate than was aniline in the presence of acetic acid.

Attempts were made to separate the amorphous precipitate produced from water solutions of glucose, aniline and acetic acid, into different fractions and to identify these.

Experimental

Glucose-anilide in Water

That the formation of the brown amorphous precipitate in aqueous solutions of glucose, aniline and acetic acid is due, (a) to the formation of glucose-anilide and (b) to the further action of the acetic acid on the compound is shown below.

(a) To a 3% aqueous solution of glucose, which had stood for 24 hr. was added 34.875 gm. of aniline (9 m.p.*) and 22.5 gm. of acetic acid (9 m.p.) and the volume was made up to 250 cc. with water. The solution was allowed to stand at room temperature (22° C.) for 1½ hr., when the rotation had fallen to its lowest negative value (−2.85°). The yellow solution was then neutralized with 6 *N* sodium hydroxide and extracted with ether to remove the excess of aniline. The solution was next distilled to dryness under diminished pressure at 30°–35° C. The white residue was dissolved in methyl alcohol, cooled with

†See also Reference (2).

*Molecular proportions.

ice, and precipitated with ether. The precipitate which formed was removed by filtration, washed thoroughly with anhydrous ether and dried in a vacuum. There was thus obtained 31.71 gm. of a mixture of sodium acetate and glucose-anilide. That the latter was present was shown by the formation of tribromoaniline. Thus a solution of 0.6 gm. of the white precipitate gave, on treatment with Vaubel's reagent, 0.272 gm. of tribromoaniline of m.p. 115°-116° C. From this and a similar experiment it was calculated that the mixture contained 1.3 gm. of glucose-anilide or 12.2% of the weight theoretically possible.

(b) Glucose-anilide was prepared by heating together glucose, aniline and alcohol, according to Sorokin's (8) directions. The raw product was recrystallized four times by solution in methyl alcohol and precipitation with anhydrous ether. There was thus obtained a 29% yield of pure glucose-anilide.

A 100-cc. portion of an aqueous solution of 4.25 gm. of glucose-anilide, and 1 gm. of acetic acid at room temperature (22°-23° C.) became yellow in two days and a brown precipitate had just started to form. This precipitate gradually increased in amount and the solution remained yellow in color. In 100 days this brown precipitate was filtered, dried and weighed. Its weight was 0.06 gm. On heating it darkened and started to shrink between 140°-142° C., charred and expanded between 200°-202° C. and at 250° C. it was still expanding but did not melt.

On the other hand 50 cc. of an aqueous solution of 2.125 gm. of glucose-anilide at room temperature (22°-23° C.) was still colorless after four days and after eight days only a slight yellow color had appeared. In 100 days the solution was still light yellow and no precipitate had formed. The solution reduced Fehling's solution at that time.

The effect of acetic acid on the rotation of glucose-anilide in aqueous solution was also observed, as given in Table I. Time was computed from contact of the glucose-anilide with the water.

TABLE I
ROTATION OF POLARIZED LIGHT BY SOLUTION OF ACETIC ACID AND GLUCOSE-ANILIDE
IN WATER, AFTER VARIOUS INTERVALS OF TIME*

Time	35 min.	50 min.	1 hr.	2 hr.	2.5 hr.	6 hr.	1 day	2 days	7 days
<i>No acetic acid added</i>									
Rotation, degrees	-2.23	-2.66	-2.84	-3.23	-3.30†	-2.92	-2.10	-1.40	-1.18
<i>0.5 gm. (1 m.p.) Acetic acid added</i>									
Rotation, degrees			-2.32†	-0.26	-0.22	-0.19	-0.15	-0.17	-0.08

*Glucose anilide, 2.125 gm.; water to 50 cc.; tube, 10 cm.; temperature, 22° to 23°C.

†Maximum value observed, only the more pertinent readings recorded.

In connection with Table I, it might be pointed out that when there was no acid present, the rotation of the solution of glucose-anilide gradually became

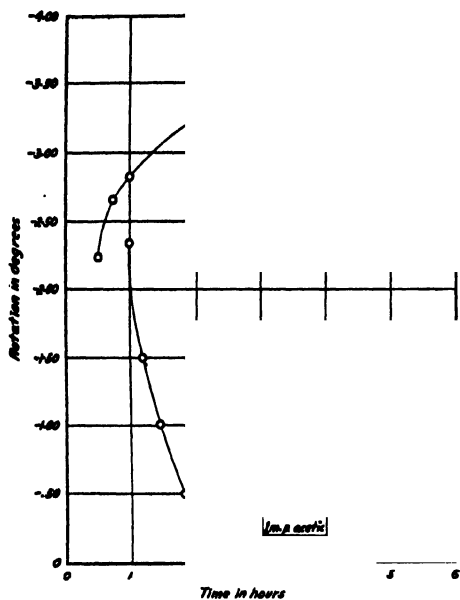


FIG. 1. Effect of acetic acid on the rotation of glucose-anilide in water solution.

in the presence of acid. Not only is this true but the rotation quickly becomes less negative when acid is present; this is doubtless due, in part at least, to hydrolysis of the glucose-anilide. The effect of acid on the rotation of glucose-anilide is clearly shown in Fig. 1.

Glucose, Aniline and Acetic Acid

The following experiments show the effect of increasing the concentration of aniline and acetic acid on the formation of glucose-anilide. The reactions were followed by means of the polariscope.

Three aqueous solutions containing 7.5 gm. of glucose were made up and allowed to stand for 24 hr. so that the rotation might become constant. To the first of these was added 2.5 gm. (1 m.p.) of acetic acid and 3.875 gm. (1 m.p.) of freshly distilled aniline; to the second 7.5 gm. (3 m.p.) of acetic acid and 11.625 gm. (3 m.p.) of freshly distilled aniline; and to the third 22.5 gm. (9 m.p.) of acetic acid and 34.875 gm. (9 m.p.) of freshly distilled aniline. The solutions were then made up to 250 cc. with water, thoroughly mixed, and filtered into 10-cm. polariscope tubes. Readings

more negative; according to Irvine and Gilmour (7) this is due to the conversion of the α - to the β -isomer. The maximum negative value (-3.30°) was recorded in 2.5 hr.; after that time the change in rotation was probably due to hydrolysis. Using the maximum negative value, -3.30° , the specific rotation for glucose-anilide in water was calculated to be $[\alpha]_D^{22} = -77.6^\circ$.

Irvine and Gilmour record a specific rotation of -77.7° for glucose-anilide in water, but 93.5° when a trace of acid was added. It was found here that when acid was present, the maximum negative value, -2.32° , was recorded one hour from the time of adding water or in 10 min. from the time that the acetic acid was added. This supports the evidence of Irvine and Gilmour that the α -isomer is very rapidly changed to the β -isomer

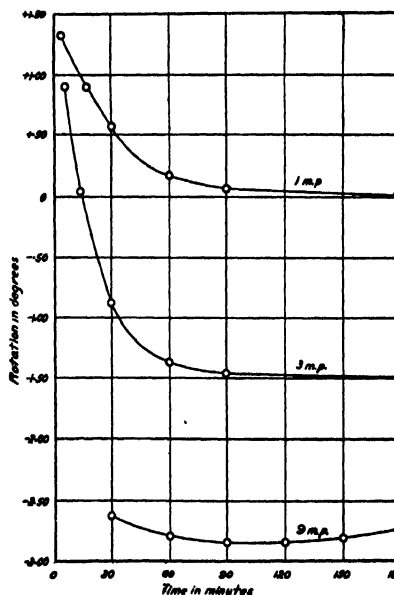


FIG. 2. Effect of increasing concentration of acid and aniline on rotation.

of the rotation were taken as shown in Table II, only the more pertinent observations being recorded. Time was computed from the addition of the aniline in all cases. The results are shown graphically in Fig. 2.

TABLE II
EFFECT OF INCREASE IN CONCENTRATION OF ANILINE AND ACETIC ACID
ON THE PRODUCTION OF GLUCOSE-ANILIDE*

Time	5 min.	15 min.	30 min.	1 hr.	1.5 hr.	3 hr.	6 hr.	24 hr.	2 days
<i>Aniline, 1 m.p.; acetic acid, 1 m.p.</i>									
α Color	+1.30 Colorless	+0.91	+0.54	+0.17	+0.07	+0.02 Light yellow	+0.01 Light yellow	-0.01† Light yellow	+0.00 Ppt'n started
<i>Aniline, 3 m.p.; acetic acid, 3 m.p.</i>									
α Color	+0.90 Colorless	+0.03	-0.87	-1.38 Light yellow	-1.46	-1.49† Ppt'n started	-1.47		
<i>Aniline, 9 m.p.; acetic acid, 9 m.p.</i>									
α Color	? Cloudy		-2.64 Light yellow	-2.78 Light yellow	-2.85†	-2.77	-2.70	? Yellow oil separated	? Dark oil separated

*Glucose, 7.5 gm.; water to 250 cc.; tube, 10 cm.; temperature, 24° C.

†Maximum value observed.

Due to the presence of the acetic acid, the preliminary formation of the α -isomer was not observed, the rotation rapidly falling to its maximum negative value. The effect of increasing the concentration of aniline and acetic acid on the production of glucose-anilide is very noticeable; for example, using 1 m.p. of aniline and 1 m.p. of acetic acid the maximum value (-0.01°) was not reached until 24 hr. had elapsed, while in the case of the solution containing 3 m.p. the maximum value (-1.49°) was attained in 3 hr. In the case of that containing 9 m.p. the maximum value (-2.85°) was reached in $1\frac{1}{2}$ hr. (Due to cloudiness, the rotation of this solution could not be read until 30 min. had elapsed.) The slight increase in rotation, which was observed in all three solutions after the maximum value had been reached, was probably due in part to hydrolysis. The above values indicate that an increase in the concentration of aniline and acetic acid speeds up the formation of the β -glucose-anilide from the α -glucose-anilide. The β -glucose-anilide is then the final product, which starts to hydrolyze as soon as the maximum negative rotation has been reached.

Determination of Aniline

Vaubel's volumetric method (9) for the determination of aniline was selected for the purpose. This method can be used to determine aniline either in the free form or in the anilide. The data obtained for the determination of aniline in glucose-anilide are shown in Table III.

TABLE III
DETERMINATION OF ANILINE IN AQUEOUS SOLUTION OF GLUCOSE-ANILIDE

Time, days,	First titration, cc.	Second titration, cc.	First ppt'n, gm.	Second ppt'n, gm.	Melting point, °C.
0	34.27	34.32	0.2401	0.2511	117-118
40	35.91	35.86	0.2796	0.2857	118-119

Theoretically the precipitate of tribromoaniline should weigh 0.2746 gm. (since 5 cc. of the glucose-anilide solution would contain 0.0775 gm. of aniline). Hence from Table III it is apparent that the aniline of glucose-anilide can be determined quantitatively in this manner; and furthermore that in 40 days the glucose-anilide had undergone no change resulting in the loss of aniline. The melting point of the precipitate was the same as that observed by others, namely 119° C.

The following experiment was performed to ascertain if an acetic acid solution of glucose-anilide behaved similarly. An aqueous solution (100 cc.) of 4.25 gm. of glucose-anilide and 1 gm. of acetic acid was made up in the usual manner. Again Vaubel's volumetric method for the determination of aniline was used, 5 cc. of the solution being used for each titration. The results obtained are shown in Table IV.

TABLE IV
DETERMINATION OF ANILINE IN ACETIC ACID SOLUTION OF GLUCOSE-ANILIDE*

Time, days	Number of cc. of <i>N</i> /6 sodium bromate used in:		
	First titration, cc.	Second titration, cc.	Average, cc.
0	26.98	26.88	26.93
20	19.32	19.27	19.30
40	14.79	14.76	14.78

*Glucose-anilide, 5 cc. diluted to 200 cc. with water; potassium bromide (20%), 10 cc.; hydrochloric acid (conc.), 5 cc.; indicator, starch-potassium iodide solution.

On the first day the average of the two titrations was 26.93 cc. of *N*/6 sodium bromate. When 40 days had elapsed the average of the two titrations was 14.78, just about one-half of the first reading.

These last two experiments have shown very definitely that the aniline in an aqueous solution of glucose-anilide does not change with time and that in acetic acid solution there is a marked decrease of aniline, either in the free state or in the form of the glucose-anilide.

Determination of Glucose

As solutions of glucose, aniline and acetic acid in alcohol fail, after some time, to reduce Fehling's solution, it is evident that glucose and glucose-anilide must disappear. In these experiments Bertrand's method (1) for the estimation of glucose was used to follow the change in concentration of the carbohydrate.

The data for the determination of glucose in glucose-anilide in water are shown in Table V.

TABLE V
DETERMINATION OF GLUCOSE IN AQUEOUS SOLUTION OF GLUCOSE-ANILIDE*

Time	Average of titrations, cc.	Copper, mgm.	Glucose in 2 cc., mgm.
20 min.	11.00	110.00	58.4
12 days	11.12	111.20	59.0
55 days	10.85	108.50	57.5

*Glucose-anilide, 1.0625 gm.; water to 25 cc.; 1 cc. permanganate was equivalent to 10 mgm. of copper; temperature, 21° to 22° C.

Theoretically 2 cc. of the glucose-anilide solution used in the determinations of Table V would be equivalent to 60 mgm. of glucose.

Table V shows clearly that glucose in glucose-anilide can be determined as such, and that in an aqueous solution there is no appreciable change in the concentration of the glucose in 55 days. The determinations of the glucose-anilide in acetic acid solutions are shown in Table VI.

TABLE VI
DETERMINATION OF GLUCOSE IN ACETIC ACID SOLUTION OF GLUCOSE-ANILIDE*

Time	Average of titrations, cc.	Copper, mgm.	Glucose in 2 cc., mgm.
5 min.	10.78	107.80	57.1
4 days	10.80	108.00	57.2
12 days	10.21	102.10	53.9
20 days	9.75	97.50	51.2
40 days	8.58	85.80	44.6
55 days	7.60	76.00	39.2

*Glucose-anilide, 4.25 gm.; acetic acid, 1.00 gm. (1 m.p.); water to 100 cc.; 1 cc. permanganate was equivalent to 10 mgm. of copper; temperature, 22° to 23° C.

It is quite evident from Table VI that the glucose of glucose-anilide in acetic acid solution does disappear gradually. In 55 days there was a loss of 17.9 mgm. or approximately 30%. The effect of acetic acid on the change in glucose-anilide is quite marked.

To show the effect of increasing the concentration of aniline and acetic acid on the change in concentration of glucose in the reaction between glucose, aniline and acetic acid, the following experiments were performed.

Three aqueous solutions of 7.5 gm. of glucose were made. To the first of these was added 2.50 gm. (1 m.p.) of acetic acid and 3.875 gm. (1 m.p.) of freshly distilled aniline; to the second, 7.5 (3 m.p.) of acetic acid and 11.625 gm. (3 m.p.) of freshly distilled aniline; and to the third, 22.5 gm. (9 m.p.) of acetic acid and 34.875 gm. (9 m.p.) of freshly distilled aniline. The solutions were then made up to 250 cc. with water, thoroughly mixed, and determinations of glucose in each of the three solutions were made using Bertrand's (1) method. The results of the determinations are shown in Table VII, only the more

pertinent observations being recorded. Blanks were made up in the same manner and the results are shown in Table VIII.

TABLE VII
DETERMINATIONS OF GLUCOSE IN ACETIC ACID SOLUTIONS OF GLUCOSE AND ANILINE*

Time	5 min.	6 days	12 days	24 days	40 days	65 days	90 days	110 days	140 days
<i>Aniline, 1 m.p.; acetic acid, 1 m.p.</i>									
Glucose in 2 cc., mgm.	56.2	56.2	56.2	56.1	52.9†	43.6	34.8	30.8	24.2
<i>Aniline, 3 m.p.; acetic acid, 3 m.p.</i>									
Glucose in 2 cc., mgm.	56.2	57.3	57.7	54.6†	44.0	26.4	10.8	8.0	6.3
<i>Aniline, 9 m.p.; acetic acid, 9 m.p.</i>									
Glucose in 2 cc., mgm.	58.2	49.1†	27.0	10.0	6.2	6.2			

*Glucose, 7.5 gm.; water to 250 cc.; temperature, 22° to 23° C.

†First appreciable change.

TABLE VIII
DETERMINATION OF GLUCOSE* IN ACETIC ACID SOLUTIONS CONTAINING NO ANILINE

Time	Acetic acid present, m.p.		
	1	3	9
	Glucose in 2 cc., mgm.		
5 min.	55.4	57.2	57.1
24 days	56.1	56.3	56.8
55 days	56.2	56.1	55.2

*Glucose, 7.5 gm.; water to 250 cc.; temperature 22° to 23° C.

As shown by Table VII, the effect of increasing the concentration of aniline and acetic acid on the disappearance of glucose in the reaction mixture, glucose-aniline-acetic acid is very noticeable. The solution containing 1 m.p., for example, did not show any appreciable change in concentration until the fortieth day, while in the case of that containing 3 m.p., the change was quite marked in 24 days, and in the case of that containing 9 m.p. there was a decided change in concentration in six days. The blanks showed no appreciable change in concentration of glucose over a period of 55 days. Thus it is very evident that the glucose does disappear from the reaction mixture as such, and that an increase of concentration of aniline and acetic acid has a very marked effect on the rate of disappearance. The results are shown graphically in Fig. 3.

To show the effect of temperature on the change in concentration of glucose in the reaction between glucose, aniline and acetic acid, the following experiment was performed.

To an aqueous solution of 7.5 gm. of glucose (3%) were added 22.5 gm. (9 m.p.) of acetic acid and 34.875 gm. (9 m.p.) of aniline. Water was added to 250 cc. Five minutes after the solution was made up it was placed on a water bath which was kept at the boiling point throughout the whole experiment. At intervals a portion of the solution was removed and the concentration of glucose determined. The data obtained are shown in Table IX which shows that at 97° C. the rate of disappearance of glucose is extremely rapid. In one hour 22.4 mgm. of glucose had disappeared or approximately 40%; in two hours a loss of 32.7 mgm. was observed or approximately 60%; and in 7 hours 43.5 mgm. or approximately 75%. The results are shown graphically in Fig. 4.

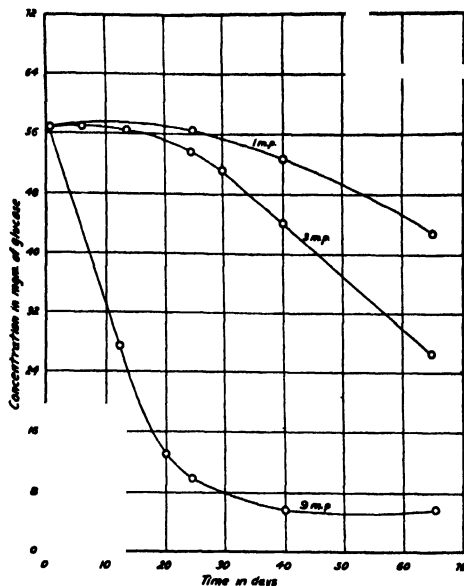


FIG. 3. Effect of increase in concentration of aniline and acetic acid on the disappearance of glucose.

TABLE IX
GLUCOSE IN GLUCOSE, ANILINE AND ACETIC ACID SOLUTION AT HIGH TEMPERATURE*

Time	Average of titrations, cc.	Copper, mgm.	Glucose in 2 cc., mgm.
5 min.	10.78	107.8	57.1
1 hr.	6.78	67.8	34.7
2 hr.	4.85	48.5	24.4
3 hr.	3.70	37.0	18.4
5 hr.	2.85	28.5	14.1
7 hr.	2.75	27.5	13.6

*Temperature, 97° to 98° C.; glucose, 7.5 gm.; water to 250 cc.; aniline, 9 m.p.; acetic acid, 9 m.p.

A Reactive Molecule

It is found that solutions of glucose, aniline and acetic acid, which contain glucose-anilide, show a reactivity greater than that of each of the components. In general such acid solutions reduce oxidizing agents more readily than corresponding blanks. Thus, in such solutions of glucose, aniline and acetic acid, two days old, the aniline is more readily oxidized to quinone by potassium dichromate than is aniline in the presence of acetic acid. Again, these acid solutions give Seliwanoff's reaction. This might show that fructose was formed but, as has been pointed out recently (6), Seliwanoff's reaction "may simply indicate the presence of a reactive sugar." These solutions also reduce potassium permanganate.

It is believed, then, that the glucose-anilide, especially in acid solution, is changed to a more reactive form, and it is suggested that this is the glucose anil,



This isomer would be more reactive and would account for the greater activity of glucose-anilide in acid solutions.

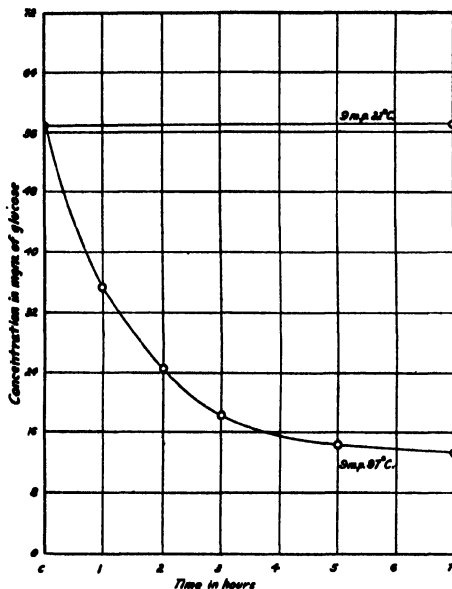


FIG. 4. Effect of temperature on the change in concentration of glucose in the reaction—glucose-aniline-acetic acid.

Solution No. 2 showed no color in 24 hr. Solution No. 3 was opaque green in three minutes but it was not until four minutes had passed that the yellow color of quinone could be detected in the ether.

To two separate test tubes containing 10 cc. of approximately $N/50$ potassium permanganate, and 2 cc. of dilute sulphuric acid was added 1 cc. of solutions Nos. 1 and 3 (two days old) and the tubes were shaken. Solution No. 1 became brown at once and turned to a clear green color in less than 30 sec. Solution No. 3 became reddish at once but was not a clear green until one minute had elapsed.

To show definitely that glucose-anilide in the presence of acetic acid was responsible for the reactivity of solutions of glucose, aniline and acetic acid, the following experiment was performed.

An aqueous solution (20 cc.) of 0.85 gm. (1 m.p.) of glucose-anilide and 0.2 gm. (1 m.p.) of acetic acid at room temperature (22°C .) was made up in the usual manner. Five minutes after the acetic acid had been added, 2 cc. of this glucose-anilide solution was added to a test tube containing 5 cc. of approximately $0.4\text{ }N$ potassium dichromate solution, 2 cc. of dilute sulphuric acid and 10 cc. of ether, and the tube was shaken. In less than five seconds

The following experiments indicate that solutions containing glucose, aniline and acetic acid show a greater activity than comparable solutions without all three components present.

Three solutions of 50 cc. were made up as follows: (1) an aqueous solution of 1.5 gm. of glucose, 0.5 gm. of acetic acid, and 0.775 gm. of aniline; (2) a comparable aqueous solution of glucose and acetic acid; (3) a comparable aqueous solution of aniline and acetic acid.

To three separate test tubes containing 5 cc. of approximately $0.4\text{ }N$ potassium dichromate solution, 2 cc. of dilute sulphuric acid and 10 cc. of ether, was added 2 cc. of solutions Nos. 1, 2 and 3 (two days old) and the tubes were shaken. Solution No. 1 became an opaque green at once and the yellow color of quinone was observed as soon as the ether had separated (in 20 sec.).

the solution became an opaque green, which was similar to that obtained in solution No. 3 above, in three minutes. The yellow color of quinone, observed in 20 sec., was similar to that obtained in solution No. 3 in four minutes.

The Colored Material

When an aqueous solution of glucose is allowed to react at room temperature with aniline in the presence of acetic acid, an amorphous precipitate is formed. Attempts were made to separate this precipitate into different fractions and to identify these. In brief, the products obtained from these experiments were all colored, amorphous and all had indefinite melting points.

The following account describes the preparation of the colored material on a large scale.

To an aqueous solution containing 180 gm. of glucose (3%) was added 837 gm. of freshly distilled aniline (9 m.p.) and 540 gm. of acetic acid (9 m.p.), and the volume was made up to six litres with water at 23°-24° C., and allowed to stand without being disturbed for 40 days. At the end of that time the reddish-orange liquid was poured off and the black oily residue was dissolved in 375 cc. of ethyl alcohol (95%). The alcoholic solution was steam-distilled for about 14 hr. to remove the excess of aniline and the black gum-like residue was dissolved in 200 cc. of ethyl alcohol (95%). This solution was diluted with alcohol and cooled. On the addition of cold water, a colloidal precipitate was obtained. This precipitate was salted out with a little sodium chloride, filtered, washed with water, and dried in a vacuum. It weighed 63 gm., was a chocolate brown in color, and on heating, darkened at 119° C., and decomposed between 140° and 146° C. By means of Kjeldahl's method, it was found to contain 8.69% of nitrogen (average of 8.66, 8.70, 8.70).

One gram of this material was oxidized with acid permanganate. Carbon dioxide was evolved but extraction of the residual liquid with ether and benzene yielded nothing.

On oxidation with alkaline permanganate, the material at first gave a decided indol-like odor, followed by the evolution of a vapor with a decidedly pleasant odor. Again extraction of the residual solution by ether and benzene yielded nothing.

Fusion of a small amount of the amorphous brown material with zinc dust gave a small amount of oil with a decidedly unpleasant and tenacious odor. On treating this with benzoyl chloride there was obtained benzanilide only.

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THE ACTION OF HYPOCHLOROUS ACID ON ARYLIDENECYANOACETIC ACIDS¹

BY JOHN ALEXANDER McRAE² AND CLARENCE YARDLEY HOPKINS³

Abstract

The action of hypochlorous acid on *p*-methoxybenzylidenecyanoacetic acid has been found to produce *p*-methoxyphenylacetic acid. When treated in the same way, *o*-methoxybenzylidene-, veratrylidene- and piperonylidene- cyanoacetic acids give the corresponding substituted phenylacetic acids in moderately good yields. Several schemes suggesting how this transformation may occur are advanced.

o-Chlorobenzylidenecyanoacetic acid gives α -cyano- β -(2-chlorophenyl)glycidic acid but this substance could not be converted to *o*-chlorophenylacetic acid. Less definite results were obtained when the aryl group of the original acid was phenyl, *o*- and *m*-nitrophenyl, cinnamyl, furyl, 3-nitroanisyl and 6-nitropiperonyl.

o-Chlorobenzylidene-, *o*-methoxybenzylidene-, 3-nitroanisylidene-, and 6-nitropiperonylidene- cyanoacetic acids have been prepared for the first time.

Introduction

Some years ago (12) it was shown that arylidenecyanoacetic acids readily combine additively with hydrogen cyanide and later (1, 3, 14) it was shown further that they can be reduced easily to α -cyano- β -arylpropionic acids. No other additive reactions of these acids, beyond the slight reactivity which they display towards chlorine and bromine and their qualitative behavior towards sodium bisulphite, appear to have been studied. Accordingly, in view of their structure as α -cyanocinnamic acids, and the ease with which cinnamic acid reacts with hypochlorous and hypobromous acids, the authors have examined the behavior of a number of arylidenecyanoacetic acids towards hypochlorous acid, and in a few cases towards hypobromous acid also.

The arylidenecyanoacetic acids used in the present investigation are benzylidene-, *o*-chlorobenzylidene-, *o*- and *m*-nitrobenzylidene-, *o*- and *p*-methoxybenzylidene-, 3-nitroanisylidene-, veratrylidene-, piperonylidene-, 6-nitropiperonylidene-, cinnamylidene-, and furfurylidene- cyanoacetic acids. The mode of application of hypochlorous acid to these substances which was found to be most suitable was that described by Rassow and Burmeister (15), for the addition of hypochlorous acid to cinnamic acid. The arylidenecyanoacetic acid was dissolved in the equivalent quantity of dilute sodium hydroxide, an excess of sodium hypochlorite solution of known strength, made by the addition of sodium carbonate to a solution of bleaching powder, was added, carbon dioxide was then introduced slowly, and finally after some time, the mixture was acidified.

Interaction occurred in each case, but the extent to which it took place and the character of the products isolated varied considerably. The most interesting results were obtained using *o*-methoxybenzylidene-, *p*-methoxybenzylidene-, piperonylidene-, and veratrylidene- cyanoacetic acids which gave respectively, *o*-methoxyphenyl-, *p*-methoxyphenyl-, piperonyl- and veratryl-

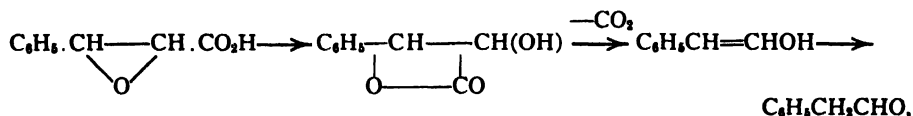
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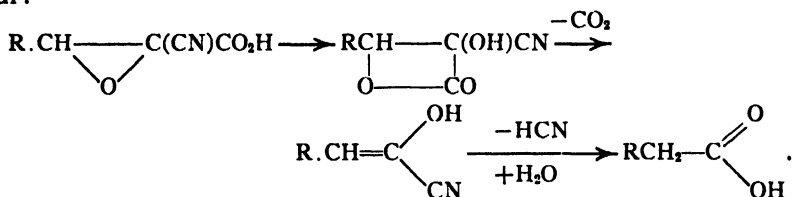
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Of these alternatives the first is the more probable on account of the ease with which phenylglycidic acid is formed from cinnamic acid chlorohydrin, and the readiness with which it is converted into phenylacetaldehyde (5). Further, phenylglyceric acid gives phenylpyruvic acid (7) and not phenylacetaldehyde when heated with hydrochloric acid. Many years ago Erlenmeyer, jr. and Lipp (8) suggested that phenylglycidic acid changed into phenylacetaldehyde through the formation of an unstable β -lactone thus:



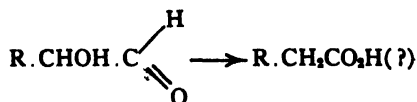
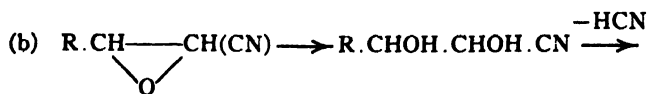
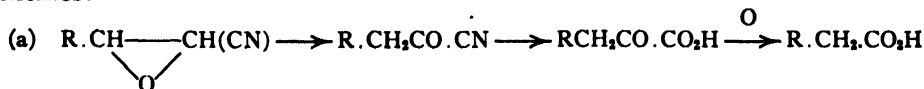
and the same suggestion may be applied here and appears plausible. That is to say, when the cyanoarylglycidic acid is released from its salt the following may occur:

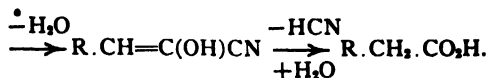
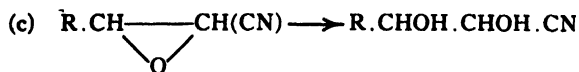


It would seem, however, that phenylglyceric acid should be equally capable of giving the hypothetical β -lactone of Erlenmeyer and Lipp, and hence should yield phenylacetaldehyde easily under the same conditions as phenylglycidic acid. Further, the formation of phenylacetaldehyde by heating dry phenylglycidic acid above its melting point must obviously follow another course.

Alternatively, it is to be expected that since phenylglycidic acid loses carbon dioxide readily when it is released from its salts, and again when it is dissolved in sodium bicarbonate (5), the α -cyanoarylglycidic acids will lose carbon dioxide with still greater ease, giving rise to arylglycidic nitrile $\text{RCH}\begin{array}{c} \diagup \text{O} \diagdown \\ \text{CH(CN)} \end{array}$. This change can probably occur in a cold alkaline

solution and the nitrile formed might react according to one of the following schemes:





Still other rearrangements may be devised but (a) has the merit that two molecular proportions of hypochlorous acid were found necessary for the production of the arylacetic acids which were obtained. However, the authors made no experiments on the effect of hypochlorites on arylpyruvic acids, but Reissert (17) has pointed out that *o*-nitrophenylpyruvic acid may be oxidized to *o*-nitrobenzaldehyde, *o*-nitrobenzoic acid or *o*-nitrophenylacetic acid, and he has shown also that *o*-nitrophenylpyruvic acid with alkaline sodium hypobromite gives a 50% yield of *o*-nitrobenzylidene bromide and with bleaching powder *o*-dinitrodibenzylidiketone was obtained in 10% yield. With *p*-nitrophenylpyruvic acid and alkaline sodium hypobromite his results were less definite, only a small amount of *p*-nitrobenzoic acid being isolated. It is possible that different effects of alkaline sodium hypochlorite on various arylpyruvic acids may account for the production, in the authors' experiments, sometimes of arylacetic acids and in other cases of acids of the benzoic acid series. On the other hand, it is doubtful if the conditions which were used in these experiments were such as to produce hydrolysis of the arylpyruvic nitriles, $\text{R} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{CN}$. Here, as in other possible schemes, data are lacking on which to base an opinion. The results obtained recently by Tiffeneau and Levy (22) showing that in the rearrangements of phenylglycidic esters, $\text{C}_6\text{H}_5\text{CH} \begin{array}{c} \diagup \quad \diagdown \\ \quad \text{O} \end{array} \text{CH} \cdot \text{CO}_2\text{R}$, at elevated temperatures the phenyl group moves



from the β - to the α -carbon, do not throw light on the results now described.

Experiments on the addition of hypobromous acid to benzylidene-, anisylidene-, and piperonylidene-cyanoacetic acids either by Berner's (4) or Read's (16) method gave back principally the unchanged acids but, in an experiment using sodium piperonylidene-cyanoacetate and sodium hypobromite, a small quantity of a neutral substance separated from the alkaline solution. This the authors were not successful in purifying completely, but it gave analytical figures in fair agreement with those required by $(\text{CH}_2\text{O})_2\text{C}_6\text{H}_5\text{CH}(\text{OH})\text{CHBrCN}$. They were unable to obtain any more of this substance in several attempts.

Experimental

A. PREPARATION OF ARYLIDENECYANOACETIC ACIDS

The method used for the preparation of the arylidenecyanoacetic acids was that of Lapworth and McRae (12). The usual condensing agent was sodium hydroxide, but in condensations using the nitro-aldehydes, aniline hydrochloride was found preferable. The acids were sufficiently pure for use in the succeeding experiments after a thorough washing with benzene. The following have been prepared for the first time.

α -Cyano- β -(2-chlorophenyl)acrylic acid, (2)-ClC₆H₄CH:C(CN)CO₂H

A solution of 25 gm. of *o*-chlorobenzaldehyde in 25 cc. of alcohol was added to a neutral solution (125 cc.) of sodium cyanoacetate made from 30 gm. of monochloroacetic acid. The mixture was made alkaline with 20 cc. of 20% sodium hydroxide and warmed with shaking until the aldehyde had reacted completely. The sodium salt of α -cyano- β -(2-chlorophenyl)acrylic acid separated on cooling and after standing an hour the mixture was strongly acidified with hydrochloric acid. The *o*-chlorobenzylidenecyanoacetic acid which separated was collected and recrystallized, first from glacial acetic acid and then from toluene from which it separated in colorless needles, m.p. 208° C. Found: N, 6.9; Cl, 16.9%. C₁₀H₆O₂NCl requires; N, 6.75; Cl, 17.1%. Equivalent: found, 207; C₁₀H₆O₂NCl requires 207.5.

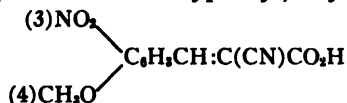
α -Cyano- β -(2-chlorophenyl)acrylic acid is readily soluble in alcohol, hot water, hot acetic acid and hot toluene. It is difficultly soluble in cold benzene and chloroform. Its sodium salt is yellow and is readily soluble in water.

Ethyl ester of α -cyano- β -(2-chlorophenyl)acrylic acid, (2)-ClC₆H₄CH:C(CN)CO₂C₂H₅

The foregoing acid was esterified by the method of Baker and Lapworth (2). After dilution of the esterification mixture it was obtained as an oil which quickly solidified. It was recrystallized from methyl alcohol from which it separated in fine colorless needles, m.p. 53° C. Found: N, 6.2; Cl, 15.2%. C₁₂H₁₀O₂NCl requires: N, 6.0; Cl, 15.1%.

 α -Cyano- β -(2-methoxyphenyl)acrylic acid, (2)-CH₃O.C₆H₄CH:C(CN)CO₂H

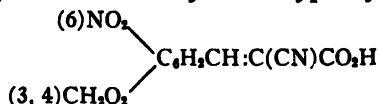
The condensation of *o*-methoxybenzaldehyde with sodium cyanoacetate takes place with great ease. If the temperature is allowed to rise too high the resulting product is an oil which is difficult to purify. The crude acid was recrystallized twice from toluene from which it was obtained in fine yellow needles, m.p. 212° C., soluble in alcohol, hot benzene and toluene. Found: equivalent, 203; N, 7.3%. C₁₁H₈O₃N requires: equivalent; 203; N, 6.9%.

 α -Cyano- β -(3-nitro-4-methoxyphenyl)acrylic acid,

3-Nitroanisaldehyde was prepared by the method of Johnson and Kohmann (11). The aniline hydrochloride method of Lapworth and McRae (12) was used for the condensation. The aldehyde (12 gm.), dissolved in 20 cc. of alcohol, was shaken with a solution of sodium cyanoacetate acid made from 10 gm. of monochloroacetic acid, to which had been added enough hydrochloric acid to liberate half of the cyanoacetic acid, and containing 5 gm. of aniline hydrochloride. On warming, condensation occurred after shaking for a few minutes and, on the addition of further hydrochloric acid, an almost quantitative yield of the acid was obtained. After three recrystallizations from glacial acetic acid the substance melted constantly at 240° C. Found: equivalent; 250; N, 11.6%. C₁₁H₈O₃N₂ requires equivalent; 248; N, 11.3%.

The substance was obtained as a white microcrystalline powder from acetic acid. It is moderately soluble in alcohol, very slightly soluble in ether and in benzene. The sodium salt is but slightly soluble in water.

α -Cyano- β -(6-nitro-3:4-methylenedioxyphenyl)acrylic acid



Piperonal was nitrated according to Fittig and Remsen (10). The method of Salway (19) in which nitric acid (sp. gr., 1.41) is employed was used unsuccessfully. The 6-nitropiperonal thus obtained was condensed with cyanoacetic acid in presence of aniline hydrochloride. Condensation took place with some difficulty and it was necessary to warm the mixture on the steam bath and to use enough alcohol to keep the aldehyde in solution. After the reaction was complete, part of the alcohol was distilled off and the product crystallized out on cooling. The crude product was recrystallized from 90% formic acid from which it separated in minute yellow crystals, m.p. 247° C., which turn brown on exposure to light. Found: equivalent, 263; N, 11.1%. $\text{C}_{11}\text{H}_6\text{O}_6\text{N}_2$ requires equivalent, 262; N, 10.7%.

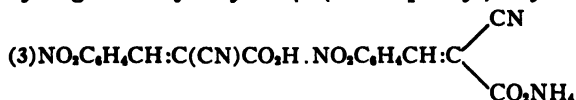
The substance is soluble in alcohol and hot acetic acid but does not crystallize well from these solvents.

Preparation of o- and m-nitrophenylcyanoacrylic acids

Attempts to condense *o*-nitrobenzaldehyde and sodium cyanoacetate in presence of sodium hydroxide did not produce the desired acid but, using the aniline hydrochloride method, reaction readily occurred and *o*-nitrophenylcyanoacrylic acid was obtained in good yield. After five recrystallizations from glacial acetic acid, the pure α -cyano- β -(2-nitrophenyl)acrylic acid was obtained with a melting point of 231°-232° C. Other melting points recorded previously are as follows: 226° C., Fiquet (9); 223° C., Riedel (18); 223° C., Sudborough and Lloyd (20). The melting point recorded here was confirmed later by Mr. W. E. Barker in these laboratories.

Similarly, attempts to make α -cyano- β -(3-nitrophenyl)acrylic acid by condensing *m*-nitrobenzaldehyde and sodium cyanoacetate in alkaline solution gave a very impure product but again, using the aniline hydrochloride method, a product which was easily purified was obtained in excellent yield. Several recrystallizations from benzene gave the pure acid, m.p. 171° C. Found: equivalent, 217; N, 13.0%. $\text{C}_{10}\text{H}_6\text{O}_4\text{N}_2$ requires: equivalent, 218; N, 12.8%. Fiquet (9) gave the melting point of α -cyano- β -(3-nitrophenyl)acrylic acid as 214°-216° C., Riedel (18) as 172° C. and Sudborough and Lloyd (20) as 170°-171° C.

The acid was further converted into its ethyl ester which after several recrystallizations from alcohol had a melting point of 134° C. Sudborough and Lloyd (20) give the same melting point but Riedel (18) had previously found it to be 127°-128° C.

Ammonium hydrogen salt of α -cyano- β -(3-nitrophenyl)acrylic acid

On adding ammonia to crude *m*-nitrophenylcyanoacrylic acid, a mass of colorless crystals, presumably the normal ammonium salt, separated, and after removing the mother liquor the material was recrystallized from hot water. This purified ammonium salt was then dissolved in a small volume of hot glacial acetic acid and on cooling a white powder was deposited which, after several recrystallizations from acetic acid melted at 214°-215° C. Found: N, 15.5%; $\text{C}_{20}\text{H}_{15}\text{O}_8\text{N}_5$ requires; N, 15.45%. Carrick (6) has prepared similar acid potassium and ammonium salts of phenylcyanoacrylic acids.

B. ACTION OF HYPOCHLOROUS ACID ON ARYLIDENECYANOACETIC ACIDS

Preliminary experiments showed that the method of Rassow and Burmeister (15) gave the most consistent and reproducible results.

A solution of sodium hypochlorite was made by shaking 200 gm. of bleaching powder with a 500 cc. of warm water and allowing the mixture to stand for 24 hr. Sodium carbonate (200 gm.) was then added and after standing for several hours the calcium carbonate was filtered off and the solution made up to 500 cc. The available chlorine was determined iodimetrically. The solution thus prepared contained about one mole of sodium hypochlorite per litre.

p-Methoxyphenylacetic acid from *p*-methoxybenzylidenecyanoacetic acid

p-Methoxybenzylidenecyanoacetic acid (20.3 gm. = .1 mole) was dissolved in 200 cc. of water and 40 cc. of 10% sodium hydroxide. The solution was cooled to 0° C., and mixed with a volume of sodium hypochlorite solution, also at 0° C., containing .2 mole of sodium hypochlorite. About 200 cc. was necessary. The mixture was allowed to come to room temperature, and a slow current of carbon dioxide free from hydrogen chloride was passed in for two hours. The solution was allowed to stand for two hours and finally was acidified with dilute sulphuric acid. After standing overnight, the *p*-methoxyphenylacetic acid which had separated was collected, and the filtrate extracted with chloroform to recover a further quantity. After two recrystallizations the acid melted constantly at 85°-86° C. and did not depress the melting point of a specimen of *p*-methoxyphenylacetic acid prepared by Mauthner's method (13). Further identification was made by a determination of the equivalent. Found: equivalent; 168. $\text{C}_9\text{H}_{10}\text{O}_3$ requires; equivalent, 166. The yield was 51%.

Piperonylacetic Acid (Homopiperonylic acid)

This was made in an identical manner from piperonylidenecyanoacetic acid. The acid obtained was identified by its melting point, 127°-128° C., a mixed melting-point determination with a specimen made by Mauthner's method, by a determination of its equivalent and by an analysis. Found: equivalent, 182; C, 60.2; H, 4.2%. $\text{C}_9\text{H}_8\text{O}_4$ requires: equivalent, 180; C, 60.0; H, 4.4%. The yield varied from 40% to 50%.

o-Methoxyphenylacetic acid

This was prepared in good yield from *o*-methoxybenzylidenecyanoacetic acid. After recrystallizing from hot water it melted at 123° C. and the equivalent was 167 ($C_9H_{10}O_3$ requires 166).

Veratrylacetic acid (3,4-dimethoxyphenylacetic acid)

This was produced in 30% yields from veratrylidenecyanoacetic acid. On precipitating the acid from the reaction mixture it was obtained as an oil which was redissolved in dilute sodium hydroxide, filtered from a slight turbidity and again acidified. After removing water of crystallization, the acid was obtained in its anhydrous form, m.p. 98° C. Tiemann (21) gave the melting point as 98°-99° C. It was also identified by its equivalent. Found: equivalent, 197. $C_{10}H_{12}O_4$ requires 196.

 α -Cyano- β -(2-chlorophenyl)glycidic acid, $ClC_6H_4CH \begin{array}{c} \diagup \diagdown \\ \text{O} \end{array} C(CN)CO_2H$

o-Chlorobenzylidenecyanoacetic acid (10.3 gm. = 0.05 mole) was dissolved in 100 cc. of water and 20 cc. of dilute sodium hydroxide. The ice-cold solution was mixed with an ice-cold solution of sodium hypochlorite (0.1 mole). A rise of about 10° C. in the temperature occurred. The solution was treated as in the case of *p*-methoxybenzylidenecyanoacetic acid. Addition of sulphuric acid produced a white crystalline precipitate which weighed 5.1 gm. It was recrystallized from toluene several times and was found to have a melting point of 159° C., although some difficulty was experienced in obtaining a constant melting point. Found: equivalent, 228; N, 6.53; Cl, 16.1%. $C_{10}H_6O_3NCl$ requires: equivalent, 223.5; N, 6.27; Cl 15.9%.

It crystallizes in small needles from hot toluene or chloroform. Warmed with 20% sodium hydroxide it gives *o*-chlorobenzaldehyde. It does not immediately reduce alkaline permanganate.

 α -Cyano- β -(2-chlorophenyl)glyceric acid, $ClC_6H_4 \cdot CH(OH) \cdot C(OH)(CN)CO_2H$

The foregoing glycidic acid was treated with alkaline hydrogen peroxide in the hope of converting it into *o*-chlorophenylacetic acid. The glycidic acid (2.2 gm.) was dissolved in 10 cc. of alcohol made slightly alkaline with sodium hydroxide and to this there was added 30 cc. of 10% hydrogen peroxide. After standing for 48 hr. the mixture was acidified and after a short time 1.6 gm. of an acidic substance separated. After several recrystallizations from methyl alcohol it melted at 186° C. Found: equivalent, 244, 246; N, 6.0; Cl, 14.7%. $C_{10}H_8O_4NCl$ requires: equivalent, 241.5; N, 5.8; Cl, 14.7%.

The substance is readily soluble in hot methyl and ethyl alcohols and also in acetone and acetic acid.

With all of the other acids used indefinite results were obtained. The procedure adopted was in general the same as that described for *p*-methoxybenzylidenecyanoacetic acid, unless the slight solubility of the sodium salts of some of the nitro-acids necessitated using more dilute solutions. Many experiments on benzylidenecyanoacetic acid gave in general: (1) gummy or resinous substances, (2) an oil having acidic properties which resisted all

attempts to purify it, (3) benzoic acid, (4) traces of a substance of melting point 97° - 98° C., apparently not phenylacetic acid, (5) the acid sodium salt of α -cyano- β -phenylacrylic acid, m.p. 197° - 198° C. Cinnamylidenecyanoacetic acid gave oily products and 20% of the acid was recovered unchanged. Furfurylidenecyanoacetic acid, which is readily made by the alkaline condensation of furfural and sodium cyanoacetate, seemed to react vigorously when a solution of its sodium salt was mixed with sodium hypochlorite but, on acidification, half of the original acid was obtained and extraction of the filtrate with ether gave a black gum.

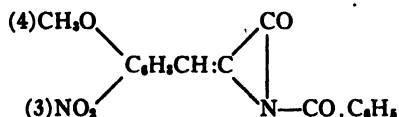
With 6-nitropiperonylidenecyanoacetic acid very little action took place and most of the acid was recovered. On the other hand, *o*-nitrobenzylidene- and *m*-nitrobenzylidene-cyanoacetic acids and 3-nitroanisylidenecyanoacetic acid seemed to react to a very considerable extent but, besides unchanged acids, only *m*-nitrobenzoic acid and 3-nitroanisic acid were isolated and these in relatively small proportions.

Action of Hypobromous Acid on Piperonylidenecyanoacetic Acid

Piperonylidenecyanoacetic acid (10.9 gm. = 0.5 mole) was suspended in 300 cc. of water and neutralized with dilute sodium hydroxide. The resulting paste was added to a solution of 0.1 mole of sodium hypobromite prepared in the usual way and the mixture stirred for 45 min. This is the procedure used by Berner (4) in preparing cinnamic acid bromohydrin. The sodium piperonylidenecyanoacetate did not dissolve. Carbon dioxide was then passed in for two hours and during this time the sodium salt appeared to dissolve and another substance separated. This was collected (4 gm.) and recrystallized from alcohol. The filtrate on acidification gave 7 gm. of unchanged acid.

The substance deposited from the alkaline solution was obtained in white flakes, m.p. 106° C. Cold sodium hydroxide did not affect it, but it dissolved on heating. Efforts to obtain more of the substance failed. It was thought to be α -bromo- β -hydroxy- β -piperonylpropionic nitrile, $(\text{CH}_2\text{O})_2\text{C}_6\text{H}_3\cdot\text{CH}(\text{OH})\cdot\text{CHBr}\cdot\text{CN}$, but the analyses that could be done with the amount available, (which was probably not quite pure) support this only in part. Found: C, 47.7; H, 2.36; N, 5.6; Br, 36.7, 36.9%. $\text{C}_{10}\text{H}_8\text{O}_3\text{NBr}$ requires: C, 44.4; H, 2.96; N, 5.2; Br, 29.6%.

Anhydride of α -Benzoylimino- β -(3-nitro-4-methoxyphenyl)acrylic acid



It was anticipated that 3-nitroanisylidenecyanoacetic acid with hypochlorous acid would yield 3-nitroanisylacetic acid. An attempt was made to prepare this acid by Mauthner's method but only the above azlactone stage was realized. The azlactone when subjected to the action of sodium hydroxide and hydrogen peroxide gave a tarry material.

3-Nitroanisaldehyde (6 gm.) was condensed with hippuric acid (6 gm.)

by heating for two hours on the steam bath with 2.5 gm. of fused sodium acetate and 10 cc. of acetic anhydride. The yellow product was filtered and washed with alcohol. It was recrystallized from hot benzene in which it is moderately soluble. It was obtained as a yellow powder, m.p. 206°C . Found: N, 8.5%. $\text{C}_{17}\text{H}_{10}\text{O}_6\text{N}_2$ requires N, 8.3%.

m-Nitrophenylsuccinic Acid, $m\text{-NO}_2\cdot\text{C}_6\text{H}_4\text{CH}(\text{CO}_2\text{H})\cdot\text{CH}_2\cdot\text{CO}_2\text{H}$

In order to test the reactivity of the ethylene linkage in *m*-nitrobenzylidenecyanoacetic acid its behavior towards hydrogen cyanide was studied. The procedure adopted was that used by Lapworth and McRae (12) in similar cases. A mixture of a solution of the sodium salt of *m*-nitrobenzylidenecyanoacetic acid (1 mole) and potassium cyanide (2 moles), to which acetic acid (1 mole) had been added, was allowed to stand for two days, acidified and evaporated almost to dryness. The crystalline product which separated was collected and hydrolyzed by allowing it to stand 24 hr. with ice-cold, fuming hydrochloric acid and then heating the mixture until the evolution of carbon dioxide ceased. The *m*-nitrophenylsuccinic acid obtained was recrystallized from hot water. The yield was 37%. The acid crystallized in colorless needles, m.p. 214°C . Found: equivalent, 120; N, 5.9%. $\text{C}_{10}\text{H}_8\text{O}_6\text{N}$ requires: equivalent, 119.5; N, 5.95%.

Acknowledgment

The authors express their thanks to Professor John Read, St. Andrew's University, who kindly informed them that it was not his intention to include a study of the arylidenecyanoacetic acids in his researches on hypochlorous acid addition.

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Abstracted in Chem. Centr. II, 1540-1541. 1930.

THE ALKALOIDS OF FUMARACEOUS PLANTS

I. *DICENTRA CANADENSIS*, WALP.¹

BY RICHARD H. F. MANSKE²

Abstract

A preliminary examination of *Dicentra canadensis* tubers collected in the province of Quebec has revealed the presence of protopine, which had previously been found, together with three typical alkaloids of *Corydalis* species, namely: bulbocapnine, corydine and isocorydine. The presence of the last in comparatively large quantity is surprising in view of its general scarcity elsewhere. The existence of other bases is indicated and will be made the subject of studies on a larger quantity of material.

Of the non-basic materials, the most interesting is a nitrogenous orange-colored substance, $C_{22}H_{29}O_8(OMe)_3(-CON=)_2$, the complexity of which is reminiscent of oxyacanthine or dauricine.

The natural order Fumariaceae is frequently included by botanists as a sub-order, Fumarioideae, in Papaveraceae, to expedite classification of some plants which occupy an intermediate position. For the present, however, the author proposes to retain the classification of Fumariaceae for purposes of simplicity, although the chemical constituents frequently betray a closer relationship, e.g., the widespread occurrences of protopine and allocryptopine.

Only three genera of Fumariaceae are native to northern America, namely, *Dicentra*, *Corydalis* and *Adlumia*, the last mentioned being endemic and represented only by one species, *A. fungosa*. It is the intention of the author to investigate as many of these interesting plants as time and material will permit, devoting particular attention to the alkaloidal constituents.

In regard to *Dicentra*, the literature records that the alkaloids of six species have been investigated, but only in the case of three, namely, *spectabilis* (3, 4, 7), *formosa* (2, p. 76, 3, 4) and *pusilla* (1), was this examination more than cursory. The investigations of *cucullaria* (2, 6) have yielded contradictory results and the available information in regard to *canadensis* (2, p. 76) and *eximia* (5) is incomplete. Protopine is present in all instances while dicentrine has been found only in *pusilla* and *formosa* and its presence in *canadensis* is doubtful.

In 1931 advantage was taken of an opportunity to collect a reasonable supply of *Dicentra canadensis* in the Gatineau Hill region of the province of Quebec, and the present paper records the preliminary examination of this material. At the time of collection the aerial portions of the plant had withered so that only the tubers were available. These were separated from adhering foreign matter by drying on coarse screens with occasional shaking. The dried material was then crushed between steel rolls and exhausted with methanol in a Soxhlet extractor.

Although the details of the examination of the extract are given in the experimental section it may be mentioned that dicentrine has thus far not been obtained and its presence is doubtful. Protopine is present in quantity and

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constitutes practically the entire amount of non-phenolic bases. Of the phenolic alkaloids, at least one has not been obtained in a crystalline state, while three others, namely, bulbocapnine, corydine and isocorydine, have been obtained in a pure condition and definitely identified.

The isolation of these bases is of considerable botanical interest since the only sources hitherto known are some species of *Corydalis* and in particular, *C. tuberosa* (native to Asia). The analogy however does not go too far since corytuberine, the typical alkaloid of *C. tuberosa* appears to be entirely absent. It would seem therefore that the genus *Dicentra* resembles the genus *Corydalis* most closely in *D. canadensis*, and that the approach to *Dicentra* from *Corydalis* is through *C. tuberosa*. On this basis it should be possible to arrange the species of *Dicentra* and *Corydalis* in a series such that the extremes (e.g., *D. pusilla* at one end and *C. solida* at the other) show little, if any, chemical relationship, but as the transition point is approached the relation becomes more apparent. There are not sufficient data available at present to arrange the series in detail but the botanical and geological significance in relation to evolution is abundantly evident.

The separation of bulbocapnine, corydine, and isocorydine has hitherto presented considerable difficulty, and this is perhaps the reason that isocorydine has only recently been isolated from *Corydalis* roots (11). It was observed, however, that corydine and isocorydine may be quantitatively removed from a strongly alkaline solution by extraction with ether, whereas bulbocapnine is not extracted and may be recovered by saturation with carbon dioxide or with ammonium chloride. The tedious procedure of purifying corydine hydrochloride by fractional crystallization, a procedure which tends to accumulate the impurities with the isocorydine, has also been successfully avoided. If the mixed hydrochlorides are washed with cold chloroform the isocorydine salt rapidly dissolves, and the base regenerated from the solution yields the pure substance after one or two recrystallizations from methanol. The insoluble hydrochloride may be freed of the last trace of isocorydine by washing again with several portions of hot chloroform and recrystallizing once from water. The regenerated base is readily obtained in a pure condition by recrystallizing from ether or ethyl acetate.

Of the non-basic constituents of *D. canadensis*, the coloring matter is the most interesting. Not only are the tubers and the extract intensely yellow, but most fractions are colored unless special precautions are taken. Appropriate treatment of the chloroform extract yielded the substance in question as fine orange-colored needles melting sharply at 237—238° C.* Its solution in chloroform is a beautiful orange with a delicate reddish cast.

Experimental

The air-dried and crushed tubers (1680 gm.) were exhausted in a Soxhlet extractor with methanol and the excess solvents distilled from the extract. Much water was added to the latter and the mixture heated on a steam bath until the last of the methanol had been removed. While still warm, the

*All melting points are corrected.

mixture was acidified to Congo red with hydrochloric acid and about a twentieth of its volume of chloroform added. The mixture was then allowed to cool and settle for several days and finally filtered with suction. The small amount of dark amorphous residue (*R*), the examination of which has been postponed, was adequately washed alternately with water and with chloroform. The chloroform layer of the filtrate was separated and the aqueous layer extracted with chloroform until the extract was only faintly colored. All the chloroform extracts (*E*) were combined. The dark yellow residual aqueous solution was filtered through a layer of charcoal to remove a small amount of dark insoluble product which accumulated during the extraction. The filtrate was basified with an excess of ammonia and the liberated bases removed by extraction with chloroform until the extract was no longer colored.

The solvent was largely removed from the combined extract and the concentrated solution filtered through a layer of charcoal to remove a small amount of insoluble resin and some inorganic impurities which had been carried along mechanically. The solvent was completely removed from the filtrate and the residue dissolved in hot dilute hydrochloric acid. The filtered solution (charcoal) was basified with excess potassium hydroxide, and the precipitated base filtered off and washed with water.

The dried precipitate was dissolved in chloroform and the somewhat turbid solution was filtered through charcoal. The solvent was largely distilled off and displaced by repeated evaporation with methanol. While still hot, a base crystallized from the solution in microscopic needles, frequently united to spherical aggregates. As thus obtained, the melting point was 203–204° C. and two additional recrystallizations from chloroform-methanol failed to raise this appreciably. Since it was suspected that the alkaloid in question was protopine, further purification was effected by recrystallizing the sulphate from water. The regenerated base after recrystallizing once more, was finally obtained in colorless microscopic needles melting at 206° C. Since this is only 1° C. lower than the accepted melting point of protopine and since the color reaction in concentrated sulphuric acid is that of protopine, there can be no doubt regarding the identity of the base. The analytical figures afford the final evidence. Calcd. for $C_{20}H_{19}O_5N$: C, 67.99; H, 5.38; N, 3.97%. Found: C, 68.16; H, 5.54; N, 4.51%.

Attempts to isolate other non-phenolic bases from the mother liquors of the protopine preparation were unsuccessful, but since the total yield of protopine was only about 3 gm., traces of other alkaloids may have been overlooked.

The *alkaline filtrate* from the crude protopine was exhausted with ether and the combined extracts evaporated. Subsequent examination showed that the small amount of bases obtained here consisted almost exclusively of corydine and isocorydine but, since the isolation of these bases from the chloroform extract (*E*) is later to be described, further details will be omitted here. The alkaline solution which contained no further ether-soluble portion was saturated with carbon dioxide and the precipitated bases filtered off and washed thoroughly with water. (The filtrate on re-extraction with ether yielded a further

small amount of bases from which a little bulbocapnine has been isolated, together with a trace of another base. Since a larger quantity of *D. canadensis* is in process of examination further study of this substance is postponed. For the sake of brevity it is proposed to refer to this product in future as *E E*.)

The mixture of precipitated phenolic bases was dried *in vacuo* and dissolved in chloroform. The filtered solution (charcoal) was evaporated to a small volume and treated with a large volume of ether. The brown, amorphous precipitate (*P*) was filtered off and thoroughly washed with ether and the combined filtrate and washings evaporated to a small volume, when crystallization of a pale yellow base rapidly ensued. The solid was filtered off, washed with cold methanol in which it is sparingly soluble, and recrystallized from a mixture of chloroform and methanol. The product was still pale yellow, but this color was largely removed by one recrystallization from a large volume of hot ethanol (charcoal). As thus obtained the base consists of almost colorless stout plates melting sharply at 202° C. Bulbocapnine is generally stated to melt at 198° C., presumably uncorrected. The phenolic nature of the base, together with the fact that its solution in alkali acquires a green color on exposure to the air, pointed to the suspicion that it was bulbocapnine. The analytical figures of the free base as well as the properties of the methiodide amply bear out this supposition and, finally, admixture with an authentic specimen of bulbocapnine from *Corydalis cava* caused no depression in the melting point. For the latter, the author is greatly indebted to Prof. Ernest Späth of the University of Vienna. Calcd. for $C_{19}H_{19}O_4N$: C, 70.15; H, 5.86; N, 4.31; OMe, 9.54%. Found: C, 69.84; H, 5.90; N, 4.31; OMe, 10.67, (mean of duplicate analyses).

Bulbocapnine Methiodide

A solution of the bulbocapnine obtained as described above in chloroform-ethanol was treated with an excess of methyl iodide. The crystalline product which rapidly separated was filtered off after a short time and washed with cold methanol. From hot water it crystallized in elongated needles and from hot 80% alcohol it was obtained in short stout rods, both products melting at 258° C, with decomposition. Calcd. for $C_{20}H_{22}O_4NI$: N, 3.00; I, 27.19%. Found: N, 2.98; I, 27.19%.

Examination of the Chloroform Extract E; Isolation of Corydine

The combined chloroform extract (*E*) was distilled until the distilled chloroform no longer carried water with it. The small amount of dark resin which had separated was filtered off through a layer of charcoal, and the clear, dark orange solution repeatedly extracted in a separatory funnel with 1% hydrochloric acid. This procedure was excessively tedious and only after 30 extractions (with a volume of acid equal to the chloroform solution) was the operation interrupted. The combined aqueous extract was evaporated *in vacuo* to 2500 cc., filtered through a layer of charcoal and extracted with eight litres of ether in five successive portions.

The dissolved ether was expelled from the aqueous solution by boiling and the filtered solution (charcoal) extracted with chloroform until the extracts were only pale yellow. Examination of the residual aqueous solution disclosed the presence of only protopine and bulbocapnine in tractable amounts, and since the isolation of these bases has already been described, further details need not be given again.

The combined chloroform extract was distilled until the water was removed, filtered through charcoal and evaporated to a small volume. Crystallization of a sparingly soluble hydrochloride ensued while the solution was still hot. It was filtered off and washed first with chloroform and then with methanol. Recrystallized from hot water in which it is sparingly soluble, it was obtained in colorless stout prisms which began to shrink at 240° C. and melted with charring at 258° C. Calcd. for $C_{20}H_{23}O_4N.HCl$: C, 63.57; H, 6.41; N, 3.71; OMe, 24.64; Cl, 9.38%. Found: C, 63.24; H, 6.32; N, 4.05; OMe, 24.26; Cl, 7.33% (mean of duplicate analyses).

The low value for chlorine is probably to be attributed to a slight loss of hydrogen chloride during the thorough drying of the analytical sample. The yield of purified hydrochloride was 2.3 gm.

When the aqueous solution of this salt was treated with an excess of potassium hydroxide the base was precipitated at first but readily redissolved. The strongly alkaline solution was extracted ten times with a large volume of ether, and the combined extracts evaporated somewhat and clarified over anhydrous sodium sulphate. Further evaporation gave a yield of long stout crystals which separated while the solution was still warm. The mother liquor was removed by decantation and the crystals washed with ether. As thus obtained the substance melts sharply at 124° C. and it is evidently the alcoholate of corydine described by Gadamer (8, 9, 10). The solvent of crystallization is removed only with difficulty and for this purpose a small specimen was heated in a high vacuum at 75° C. for several hours. Recrystallization from dry ethyl acetate free of alcohol yielded the pure base in stout prisms melting sharply at 148.5° C. Gadamer gives 149° C. for the dry base. Calcd. for $C_{20}H_{23}O_4N$: C, 70.38; H, 6.75; N, 4.10%. Found: C, 70.20; H, 6.98; N, 3.85%.

Corydine Methiodide

A small amount of corydine was treated with excess methyl iodide in chloroform solution. After standing for several days the solvent and excess reagent were evaporated. The residue was recrystallized twice from hot water and was obtained in colorless needles or in short stout prisms, both forms sintering at 220° C. (214—215° C. uncorr.) and decomposing completely at 228—230° C. Gadamer (9, p. 679) gives m.p. 213—214° C. (presumably uncorr.) with decomposition.

Isolation of Isocorydine

The chloroform mother liquor from which the corydine hydrochloride had crystallized was freed of chloroform by repeated evaporation to a syrup with methanol. On cooling the somewhat concentrated solution a copious yield of a crystalline hydrochloride separated. This was washed with cold methanol

and then extracted with cold chloroform. A small amount of corydine hydrochloride remained undissolved. The chloroform was removed from the extract and the residue dissolved in water. The clear filtered solution (charcoal) was basified with excess potassium hydroxide and the phenolic base removed by repeated extraction with ether. The free base rapidly crystallized as the combined extract was concentrated. The residue was dissolved in a large volume of ethyl acetate and the filtered solution (charcoal) evaporated to a small volume to which hot methanol was added. Crystallization readily ensued. After one recrystallization the free base was obtained in almost colorless deep four-sided tablets melting sharply at 184° C. (9). Calcd. for $C_{20}H_{23}O_4N$: C, 70.38; H, 6.75; N, 4.11; OMe, 27.27%. Found: C, 70.35; H, 6.78; N, 4.26; OMe, 27.50%. Mean of duplicate analyses.

The calculated analyses for methoxyl are based on three such groups, and in view of the excellent agreement between the two sets of figures there can be no question of the identity of the alkaloid as isocorydine. By working over the various fractions and the mother liquors nearly five grams of pure isocorydine was obtained. This alkaloid is therefore the largest single constituent of any base found in the tubers under investigation.

Examination of the Mother Liquor from the Crystallization of Isocorydine Hydrochloride

The methanol mother liquor, from which no more crystalline material separated, was evaporated to a brittle resin in a vacuum desiccator over solid potassium hydroxide. Solution in chloroform left a small amount of dark resinous material which was filtered off. The dark brown solution was freed of solvent and the residue dissolved in hot dilute hydrochloric acid. The solution was boiled to expel the small amount of residual chloroform and exhausted with ether, which removed a small amount of resinous material. The aqueous solution was basified with excess potassium hydroxide and a slight turbidity removed by filtration with the aid of charcoal. The dark filtrate was thoroughly exhausted with ether, which removed a small amount of corydine and isocorydine, the separation and identification of which was effected by a repetition of the procedure outlined above.

The aqueous alkaline solution was saturated with carbon dioxide and the precipitated bases filtered off and thoroughly washed with water. (The filtrate yielded to ether extraction a small amount of bulbocapnine together with a product presumably identical with that previously obtained from another fraction and referred to as *E E*.)

The thoroughly dried mixture of bases was dissolved in chloroform and a slight amount of dark insoluble residue filtered off through a layer of charcoal. The somewhat concentrated filtrate was treated with a large volume of ether and the precipitate filtered off and washed with the same solvent. Concentration of the extract yielded a small amount of bulbocapnine.

The ether-insoluble product consisted of an amorphous brittle resin, further examination of which soon disclosed its identity with a similar product previously obtained in smaller amount and referred to as (*P*).

Since this product, probably a mixture, is still under investigation, further details regarding its properties are omitted for the present.

Examination of the Non-basic Chloroform-soluble Fraction

The chloroform solution from which all basic material had been removed by exhaustive washing with dilute acid had an intense orange color. The greater portion of the solvent was removed by distillation and the remainder was allowed to escape spontaneously from the thick, dark syrupy mass. The resin thus obtained (55 gm.) was pulverized in a mortar and extracted in a Soxhlet apparatus with petroleum ether, ether, and ethyl acetate in the order named.

Although all extracts were yellow, the greater portion of the coloring matter had obviously been removed by the ethyl acetate. The latter extract was heated to expel as much ethyl acetate as possible and the residue heated for several hours with an excess of alcoholic potassium hydroxide. The alcohol was largely boiled off and the mixture diluted with much hot water.

This treatment served to remove some acidic products from the main constituent which remained as a yellow granular precipitate. The latter was filtered off and thoroughly washed with water. After air drying it was suspended in boiling methanol, the mixture cooled and the solid filtered off and washed with methanol. Recrystallization of this product was conveniently effected by solution in hot chloroform, filtering with the aid of charcoal and adding hot methanol to the concentrated filtrate. A repetition of the process yielded the coloring matter (9 gm.) in orange-colored needles, melting at 237—238° C. Calcd. for $C_{37}H_{38}O_{10}N_2$; C, 66.27; H, 5.67; N, 4.18; 3 OMe, 13.89; mol. wt., 670. Found: C, 65.96, 65.97; H, 5.52, 5.78; N, 4.29, 4.22; OMe, 14.02, 13.87; mol. wt., 494, 488 (Rast).

The calculated formula, because of its complexity, is obviously not certain, but in view of the fact that the ratio of methoxyl to nitrogen is almost exactly 3:2 there must be at least two nitrogen atoms in the molecule, and more are not permissible because of the molecular-weight determination, which is already too low. Taking into account the non-basicity of the substance it is possible to expand the formula to $C_{32}H_{25}O_5(OMe)_3(-CON=)_2$.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

II. *DICENTRA CUCULLARIA* (L.) BERNH.¹

By RICHARD H. F. MANSKE²

Abstract

A preliminary investigation of *Dicentra cucullaria* has confirmed the presence of protopine and disclosed the presence of at least three other alkaloids which have been obtained in crystalline form. The isolation of cryptopine is of interest since it had previously been obtained only from opium.

Two other bases, referred to as (α) and (β) are perhaps new but their naming is deferred until more is known concerning their nature.

The investigation is being actively pursued along the lines of the constitutional analysis of base (α) and of base (β), as well as a search for other bases in a larger quantity of plant material.

Attention is called to the high content (24.8% or more) of sucrose present in the dried tubers.

In connection with a previous communication (4) treating of *Dicentra canadensis*, a program of research dealing with plants of the N.O. Fumariaceae was outlined and in continuation, a preliminary examination of *D. cucullaria* has been carried out.

Fischer and Soell (3) working with *D. cucullaria* of Wisconsin, U.S.A., origin obtained protopine and two unidentified bases. Analyses are not recorded, but the melting points are given as 230—231° (dec.) and 215° C. respectively. Since these properties do not agree precisely with those of any alkaloids likely to be present, further examination was deemed desirable.

More recently Black, Eggleston, Kelly, and Turner (1) examined *D. cucullaria* of Virginia origin and confirmed the presence of protopine but reported in addition a new alkaloid, cucullarine, m.p. 169° C., together with amorphous bases.

Botanists have recognized but one species of *D. cucullaria* although the latter authors (1) hinted at a possible difference between those of eastern and of western habitat, and, aside from the purely chemical interest attached to a further investigation and identification of the alkaloids present, the opportunity of arriving at a botanical distinction based on chemical analysis presented itself.

The tubers of the species under consideration, which were collected in the Gatineau Hills in the province of Quebec after the aerial parts of the plants had withered, consist of a cluster of elongated ellipsoidal grains arranged about a central core of two or more less regular, but larger and symmetrically disposed, segments. The color varied from a pale ivory to a delicate pink hue, the latter being due to numerous microscopic red spots on the surface, the intensity and extent of which are influenced by the amount of sunlight to which they have been exposed. In either case the dried tubers are of a non-descript greyish amber—the original pink spots, however, are still observable with a lens.

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Chemical examination soon confirmed the presence of protopine but neither the work of Fischer and Soell nor that of Black and coworkers could be substantiated as to the other alkaloids. Cucullarine has thus far not been found and the two bases referred to by the former authors are probably absent, although that described as melting at 215°C . (corr ?) may possibly be identical with the base now isolated (m.p. 221°C .*), and which is almost certainly cryptopine. Owing to the minute quantity thus far isolated, further characterization than that afforded by micro-analysis and color reaction has not been possible.

The presence of cryptopine in plants other than *Papaver somniferum* occasions some surprise only for the reason that it has not previously been found elsewhere. Its close chemical relation to protopine, and the widespread occurrence of the latter have been the cause of some conjecture as to its restricted occurrence. It must be borne in mind, however, that cryptopine is present in opium in appreciable quantities only in rare cases (5), and its isolation from this source is occasioned largely because of the fact that enormous quantities of mother liquors are available from the commercial preparation of morphine and its associates.

Two other alkaloids, both of which appeared to be new, are also present. Both are soluble in excess of fixed alkali and may be extracted from strong hydrochloric acid solution by means of chloroform, but not by means of ether.

It is not proposed to name these alkaloids until further knowledge of them is obtained, and for the present they will be referred to as (α) and (β).

Their separation is detailed in the experimental section and it suffices to note here that base (α) was obtained in large, colorless, elongated prisms, melting sharply at 177°C . Analytical figures indicate $\text{C}_{20}\text{H}_{17}\text{O}_6\text{N}$ and methoxyl is entirely absent. Base (β) was obtained in crystalline form only by precipitation from alkaline solution by means of carbon dioxide, and analysis gives figures intermediate between $\text{C}_{20}\text{H}_{23}\text{O}_6\text{N}$ and $\text{C}_{20}\text{H}_{23}\text{O}_7\text{N}$.

Danckworttt (2, p. 613) while investigating the alkaloids of *D. spectabilis* incidentally isolated some sucrose, and the same carbohydrate was present in astonishing amounts (24.8% of air dried tubers) in tubers which were collected after the aerial portion of the plant had withered. Such a liberal store of reserve food amply accounts for the vigorous growth of the plant in early spring.

Experimental

The air-dried and thoroughly ground tubers (580 gm.) were extracted in a Soxhlet extractor with redistilled methanol and the solvent largely distilled from the extract. The latter was allowed to remain at room temperature for several weeks, during which time a copious yield of sucrose separated as a dense crystalline cake. Methanol was added to the supernatant syrup to facilitate handling, and the crystals were filtered off and washed with 90% methanol. The yield of this product (24.8%), which consisted of well-defined, slightly brown crystals, was 144 gm. and for obvious reasons represents the minimum quantity actually present. Recrystallized from 50% ethanol or from 80%

*All melting points are corrected.

methanol the sugar was obtained in colorless crystals indistinguishable under the microscope from sucrose similarly recrystallized. It reduced Fehling's solution only after hydrolysis with dilute acid. It had a sweet taste.

The combined mother liquor and washings from the sucrose was freed of the greater portion of the methanol on a steam bath, diluted with a large quantity of hot water, and boiled to expel the remaining methanol. While still warm, it was rendered acid to Congo red with hydrochloric acid, shaken with chloroform and the entire mixture filtered with suction. There remained only a negligible residue which was discarded. The chloroform was separated from the aqueous solution *A* and the latter extracted with chloroform until the extract was colorless.

Examination of the Aqueous Solution—Isolation of Protopine and Cryptopine

The aqueous solution *A* which had been exhaustively extracted with chloroform was basified with excess ammonia and the liberated bases removed by chloroform extraction. The combined extract was distilled until the water was removed and the residual solution was filtered through a layer of charcoal. The filtrate was freed of chloroform, dissolved in dilute hydrochloric acid and the filtered solution basified with excess potassium hydroxide. After remaining in the ice chest overnight, the precipitated base had assumed a granular form and was filtered off and washed with water. (The filtrate on treatment with ammonium chloride or saturation with carbon dioxide yielded a mixture of phenolic or weakly acidic bases from which no pure substance has been obtained. The yield was, however, quite small, and further examination is delayed until more material is available.)

The dried non-phenolic mixture thus obtained was dissolved in chloroform (charcoal), and the filtered solution evaporated to a thick syrupy residue. Treatment with much hot methanol and seeding with a crystal of protopine, or long standing, yielded the latter alkaloid in dense aggregates of microscopic needles, melting at 202° C. Repeated recrystallization raised this melting point only slowly, but purification of the hydrobromide recrystallized from hot water, and regeneration and crystallization of the base yielded colorless minute needles giving the color reactions of protopine. Alone or admixed with a specimen of protopine from *C. canadensis* it melted at 205—206° C.

The combined mother liquor from the isolation of the protopine was evaporated to a resin, the latter dissolved in a small volume of methanol, and the solution acidified with a slight excess of concentrated hydrogen bromide. In the course of several days crystallization of a sparingly soluble salt, which proved to be protopine hydrobromide, resulted. The mother liquor from this was diluted somewhat with water and the greater portion of the methanol distilled off. On cooling, the solution set to a gelatinous mass of minute hair-like needles. Since this was not in a form suitable for manipulation, the mixture was allowed to remain in a warm place for several days during which time the crystals became more compact. Filtration with suction and cautious washing with water and with dilute methanol served to yield a small quantity of colorless crystals. The base was prepared from this product in the usual

way and recrystallized from chloroform-methanol. It was thus obtained in well-defined, colorless, narrow plates, melting sharply at 221°C ., which melting point was not raised by recrystallization. With aqueous oxalic acid it yielded a sparingly soluble oxalate. A crystal dissolved in acetic acid and treated with sulphuric acid rapidly gave a violet coloration, which changed to green on heating to 150°C . Further characterization was not possible owing to the small amount obtained. In view of the analysis however, little doubt remains that the alkaloid is *cryptopine*. Calcd. for $\text{C}_{21}\text{H}_{23}\text{O}_5\text{N}$: C, 68.29; H, 6.23; N, 3.80; OMe, 16.80%. Found: C, 68.40; 68.31; H, 6.30, 6.28; N, 4.05, 4.06; OMe, 17.06, 17.28%.

Examination of the Chloroform Solution—Isolation of Two Unidentified Bases

The solvent was distilled from the combined chloroform extract and the residue dissolved in a small volume of methanol to which was added a little hydrochloric acid. The solution was heated to boiling and treated with 1500 cc. of boiling water. The mixture was thoroughly shaken and cooled in ice. The supernatant liquid was decanted from the residue, the latter again dissolved in methanol, and the solution treated with hot water. The combined decantate was filtered through a layer of charcoal and thoroughly extracted with ether. The ether was removed from the solution by heating, the latter basified with excess ammonia, and the precipitated bases extracted with chloroform. The combined extract was distilled until dry, filtered through a layer of charcoal and the solvent largely removed. Methanol was added to the residue and the solution set aside. In the course of several days crystallization of a base was complete. After filtering and washing with methanol, in which it was only sparingly soluble, it melted at $175\text{--}176^{\circ}\text{C}$. Recrystallized twice from chloroform-methanol it consisted of large, colorless, elongated plates, m.p. 177°C . With concentrated sulphuric acid in the cold it yielded a greenish-yellow color which on gentle warming first turned red and then developed a purple or violet cast. The yield of purified product, which is provisionally referred to as *alkaloid* α was 1.7 gm. Calcd. for $\text{C}_{20}\text{H}_{17}\text{O}_5\text{N}$: C, 65.39; H, 4.63; N, 3.82%. Found: C, 65.53, 65.48; H, 4.73, 4.70; N, 4.05, 3.92%. An attempted methoxyl determination (Zeisel) was entirely negative.

The mother liquor from alkaloid α , from which no more of the latter could be crystallized was freed of solvent, the residue dissolved in dilute hydrochloric acid and the solution filtered through charcoal. The filtrate was basified with excess potassium hydroxide and the small amount of insoluble base (mostly protopine) filtered off. The filtrate was saturated with carbon dioxide, during which process a bulky precipitate of fine acicular crystals separated. When this substance is filtered off, washed, and dried, it melts at about 204°C . It is not soluble to any appreciable extent in methanol, chloroform, ethyl acetate, or ether, either hot or cold. When it is boiled for a prolonged time under reflux with methanol it slowly but completely dissolves. It could not however be obtained in crystalline form from this solution, either by seeding or by concentration to a small volume. Further purification was therefore effected by

re-solution in dilute acid, treatment with alkali, and reprecipitation with carbon dioxide.

The precipitate was washed thoroughly with water and then with a large volume of cold methanol. The *base* (β) (or carbonate) thus obtained consisted of a colorless microcrystalline powder, melting sharply at 215° C. That it is not identical with the alkaloid reported by Fischer and Soell follows from the fact that their base was readily recrystallizable. Analysis indicated a composition intermediate between $C_{20}H_{23}O_6N$ and $C_{20}H_{23}O_7N$. If however the substance is a carbonate the formula $C_{19}H_{22}O_5N$ is in best agreement with the analytical figures, the carbonate being considered as $B_2 \cdot H_2CO_3$. Calcd. for $(C_{19}H_{22}O_5N)_2H_2CO_3$; C, 62.40; H, 6.13; N, 3.74%. Found: C, 62.91; 63.05; H, 5.99, 5.90; N, 3.72, 3.63%.

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CONDUCTIVITY DATA OF AQUEOUS MIXTURES OF HYDROGEN PEROXIDE AND ORGANIC ACIDS. II¹.

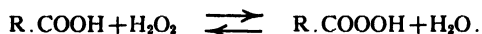
BY W. H. HATCHER² AND E. C. POWELL³

Abstract

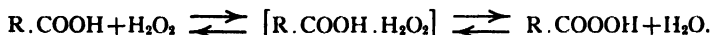
The conductivities of formic, acetic, propionic and succinic acids and succinic monoperacid have been studied in water and in aqueous hydrogen peroxide solution. These acids all add hydrogen peroxide to form un-ionized complexes which later, with time, lose water to form the so-called peracids of negligible conductivity. The formation of such complexes is dependent solely on the concentration of reagents, the peracid being produced subsequently by loss of water.

Introduction

The former contribution under this title (5) was a preliminary investigation of the ionic changes occurring when hydrogen peroxide was mixed with water as a solvent for certain organic acids. This followed as a sequel to previous studies (3, 4) which had shown two outstanding results; *viz.*, (a) that an analysis of the velocity of oxidation of an organic acid by hydrogen peroxide indicated a first-order reaction, and (b) that mixtures of water, hydrogen peroxide and an organic acid, when allowed to stand together at such a temperature as did not favor destructive oxidation, developed with time an organic peracid. The equation representing this was first formulated (4) as



The time for establishing this equilibrium and the amount of peracid obtained varied with the individual acid. The conclusions of the previous study (5) indicated that this equation should be expanded thus:—



Previously the work of Maass and coworkers (2, 6) had shown that inorganic salts dissolved in pure hydrogen peroxide were dissociated to almost exactly the same extent as in water, so that the dielectric constants of water, of hydrogen peroxide and of mixtures of these two, are almost identical. Obviously a marked change in conductivity on substituting hydrogen peroxide for water, partly or wholly, implies something other than a slight difference in degree of ionization.

In the following pages is found a careful study of these changes from beginning to end, and dealing with formic, acetic, propionic, succinic and permonosuccinic acids.

Experimental

Preparation of Reagents

The hydrogen peroxide used was that prepared in these laboratories by distillation and concentration from 30% commercial material and contained only water as impurity.

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Contribution from the Department of Chemistry, McGill University. Presented in partial fulfilment of the requirements for the degree of Ph.D.

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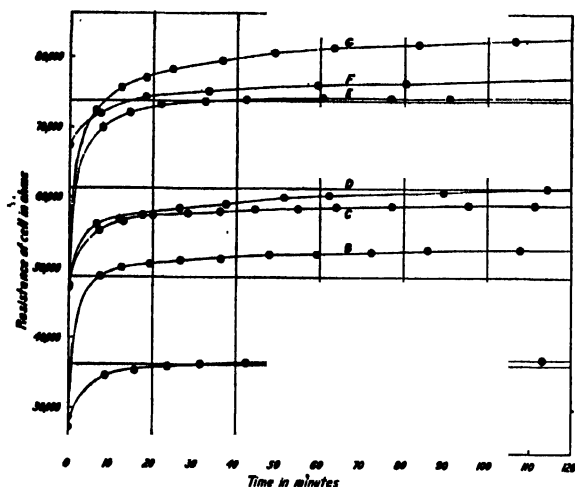


FIG. 2. Curves showing rate of increase in resistance with addition of hydrogen peroxide to aqueous organic acids. A, 25.7% formic and 10% peroxide; B, 20.6% formic and 20% peroxide; C, 15% acetic and 10% peroxide; D, 15% propionic and 10% peroxide; E, 15% acetic and 20% peroxide; F, 2.2% succinic and 4% peroxide; G, 15% propionic and 20% peroxide.

organic acids at concentrations greater than 50% within a period of ten hours. Therefore, these electrodes were inserted in the cell only while the concentration measurement was being made of the aqueous organic acid; thus they were never more than half an hour in contact with the acids. It was at first thought peculiar that, in the presence of very small amounts of hydrogen peroxide, tin electrodes remain untouched by aqueous organic acids over long periods of time.

In these conductivity measurements the reagents were separately weighed in Pyrex stoppered flasks and cooled to 0° C. in an ice-bath. They

were then thoroughly mixed in a Pyrex jacket of about 200-cc. capacity in which the appropriate cell with electrodes was at once inserted and readings

TABLE I
CONDUCTANCE OF AQUEOUS SOLUTIONS OF FORMIC ACID

Molar concentration	Sp. cond. $\times 10^4$	Molar conductivity	Molar concentration	Sp. cond. $\times 10^4$	Molar conductivity
0.228	16.61	7.285	5.874	74.00	1.260
1.122	38.42	3.425	7.120	75.66	1.063
2.281	53.85	2.361	9.648	71.71	0.743
4.639	70.36	1.517	12.357	62.15	0.503

TABLE II
CONDUCTANCE OF AQUEOUS MIXTURES OF FORMIC ACID AND HYDROGEN PEROXIDE

Molar conc'n of acid	Molar conc'n of peroxide	Sp. cond. $\times 10^4$	Molar cond.	Duration of experiment, hr.
1.076	3.081	29.73	2.763	38.0
2.851	3.129	45.20	1.586	26.0
6.104	3.216	53.64	0.879	32.0
10.091	3.323	48.71	0.483	30.0
1.112	6.365	22.42	2.017	24.0
5.412	6.580	36.54	0.675	43.0
7.141	6.701	36.10	0.505	46.0
10.484	6.887	31.05	0.296	40.0
10.011	13.551	10.40	0.104	68.0

begun. The quantities of the reagents, the volume and density being known, the concentration of each constituent was calculated in molar terms.

In Table I to Table XI and their graphic representation, it will be seen that where hydrogen peroxide is a constituent a decrease in conductivity always ensues by comparison with the purely aqueous solution; most of this decrease is apparent in the first ten minutes after mixing, with a constant value obtainable within one or two hours. But having once attained constancy, it remains at this value for many hours or days,—the length of time during which the formation of peracid required attainment of equilibrium, as shown by Holden (4) and Hill (3). The significance of these constant values will appear later.

The fact that a final value is not immediately obtained as shown in Fig. 2 is related to a temperature effect consisting of the following factors:— (a) heat of solution on mixing; (b) heat of reaction between the acid and hydrogen peroxide; and (c) a slight heating due to the mixing being carried on outside the bath.

In Tables I to XI are shown the data obtained from conductivity measurements at 0° C., first of acids and water only, and second, of acids, hydrogen

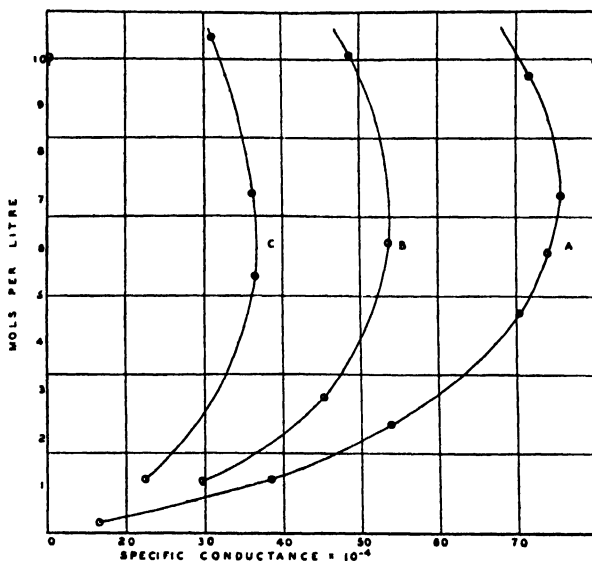


FIG. 3. Specific conductance of formic acid in water and in aqueous hydrogen peroxide. A, in water; B, in 10% peroxide; C, in 20% peroxide; \odot 40% acid and 40% peroxide.

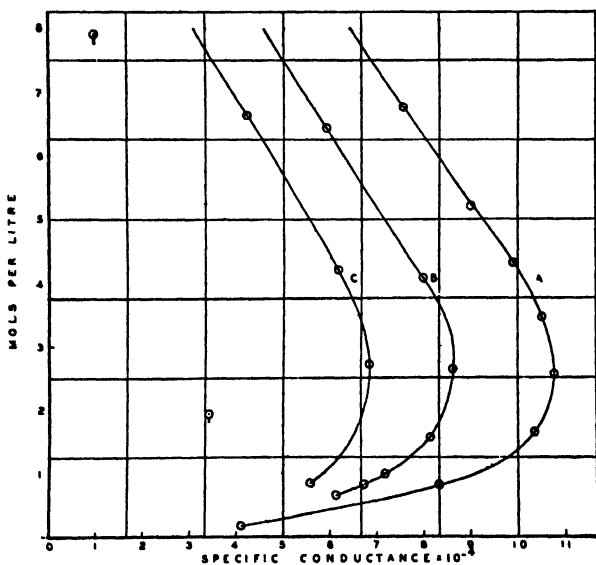


FIG. 4. Specific conductance of acetic acid in water and in aqueous hydrogen peroxide. A, in water; B, in 10% peroxide; C, in 20% peroxide; \odot in 40% peroxide.

TABLE III
CONDUCTANCE OF AQUEOUS SOLUTIONS OF ACETIC ACID

Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.	Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.
0.174	4.11	2.365	3.460	10.52	0.304
0.818	8.33	1.019	4.309	9.92	0.230
1.648	10.36	0.629	5.208	9.00	0.173
2.570	10.78	0.419	6.777	7.55	0.111

TABLE IV
CONDUCTANCE OF AQUEOUS MIXTURES OF ACETIC ACID AND HYDROGEN PEROXIDE

Molar conc'n of acid	Molar conc'n of peroxide	Sp. cond. $\times 10^4$	Molar cond.	Duration of experiment
0.633	3.065	6.13	0.969	20.5 hr.
0.810	3.069	6.73	0.831	11.0 days
0.987	3.072	7.18	0.727	16.0 hr.
1.567	3.086	8.15	0.520	12.5 days
2.633	3.106	8.63	0.328	8.5 days
3.147	3.114	8.61	0.274	24.0 hr.
4.079	3.136	7.99	0.196	24.8 hr.
6.437	3.181	5.92	0.092	14.0 days
0.835	6.336	5.58	0.669	11.2 hr.
2.712	6.413	6.86	0.253	8.0 hr.
4.200	6.472	6.17	0.147	3.8 hr.
6.640	6.571	4.21	0.063	7.25 days
1.933	13.651	3.42	0.177	42.0 hr.
7.900	13.951	0.99	0.013	38.0 hr.

TABLE V
CONDUCTANCE OF AQUEOUS SOLUTIONS OF PROPIONIC ACID

Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.	Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.
0.416	5.29	1.270	4.088	5.34	0.131
1.362	7.45	0.547	5.429	3.72	0.069
2.722	6.87	0.252	6.785	2.31	0.034

TABLE VI
CONDUCTANCE OF AQUEOUS MIXTURES OF PROPIONIC ACID AND HYDROGEN PEROXIDE

Molar conc'n of acid	Molar conc'n of peroxide	Sp. cond. $\times 10^4$	Molar cond.	Duration of experiment
0.701	3.054	5.17	0.737	45.0 hr.
2.106	3.059	5.71	0.271	17.0 days
3.938	3.064	4.12	0.105	22.0 hr.
5.636	3.069	2.52	0.045	64.0 hr.
0.724	6.309	4.01	0.554	3.0 days
2.175	6.318	4.24	0.195	26.0 hr.
4.068	6.329	2.85	0.070	68.0 hr.
5.821	6.340	1.58	0.027	28.0 hr.

peroxide and water. Measurements were made with the latter at different intervals of time during the growth of the peracid, tabulating only the total time the reaction was followed. The data given for succinic monoperacid and water were obtained indirectly, the conductivity of a mixture of known concentrations of succinic acid and succinic monoperacid being measured directly and from this value the conductivity of succinic acid subtracted. Aqueous solutions of *n*-butyric acid exhibited a decided polarization in the determination of their conductivity; a phenomenon not encountered with formic, acetic and propionic acids. Apparently, *n*-butyric acid does not form a true solution with water at 0° C.

If the specific conductivity of a 40% solution of formic acid be plotted against increasing concentration of hydrogen peroxide, a curve is obtained which resembles that for acetic acid in Fig. 5.

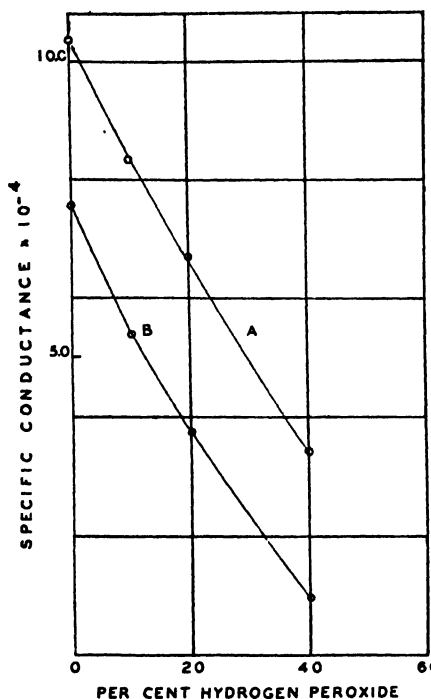


FIG. 5. Specific conductance of acetic acid with increasing content of hydrogen peroxide. A, in 10% acid; B, in 40% acid.

TABLE VII
CONDUCTANCE OF AQUEOUS SOLUTIONS OF SUCCINIC ACID

Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.	Molar conc'n	Sp. cond. $\times 10^4$	Molar cond.
0.043	3.64	8.438	0.152	6.85	4.508
0.085	5.17	6.066	0.202	7.82	3.876
0.119	6.07	5.094	0.227	8.24	3.638

TABLE VIII
CONDUCTANCE OF AQUEOUS MIXTURES OF SUCCINIC ACID AND HYDROGEN PEROXIDE

Molar conc'n of acid	Molar conc'n of peroxide	Sp. cond. $\times 10^4$	Molar cond.	Duration of experiment
0.128	0.297	6.17	4.814	25.0 hr.
0.043	0.594	3.55	8.299	45.0 hr.
0.068	0.594	4.47	6.528	13.25 hr.
0.129	0.596	6.08	4.724	11.2 hr.
0.189	0.597	7.30	3.858	54.0 hr.
0.043	1.197	3.45	8.001	46.0 hr.
0.069	1.196	4.34	6.300	4.0 days
0.130	1.199	5.90	4.554	15.25 hr.
0.191	1.204	7.05	3.698	8.0 hr.
0.194	2.448	6.63	3.420	65.0 hr.

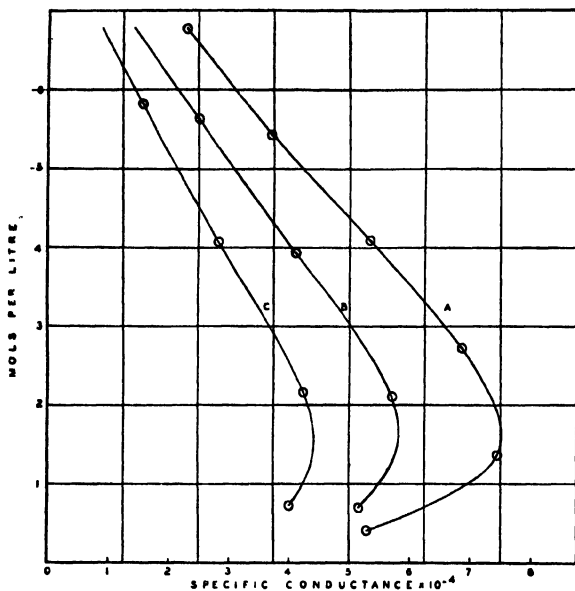


FIG. 6. Specific conductance of propionic acid in water and in aqueous hydrogen peroxide. A, in water; B, in 10% peroxide; C, in 20% peroxide.

Succinic monoperacid can be titrated in aqueous solution with sodium hydroxide using phenolphthalein as an indicator. The end-point, however, when reached tends to show fading which is due to a slow hydrolysis of the peracid radical into acid and hydrogen peroxide. The actual values obtained are shown below.

A sample (0.1000 gm.) of the succinic and monoperacid product on analysis with potassium iodide and sodium thio-sulphate showed 0.0730 gm. of succinic monoperacid and 0.0270 gm. of succinic acid. With 0.0206 *N* sodium hydroxide neutralization would require 22.28 cc. for the succinic acid alone and either 52.93 or 26.46 cc. depending on whether the peracid possessed two or one replaceable hydrogen atoms, making

If the molar conductivity be plotted against concentration of succinic acid and succinic monoperacid from the data appearing in Tables VII, VIII, IX and X, curves are obtained which are as similar to those in Fig. 8 as the curves in Figs. 3, 4 and 6 are to those in Fig. 7.

Aqueous solutions of succinic monoperacid are found to be exceedingly stable by comparison with the monobasic organic peracids. Conductivity measurements at 0° C. indicated no hydrolysis within a period of 25 hr. Moreover, decomposition in the presence of platinum black was found to be extremely slow at room temperature.

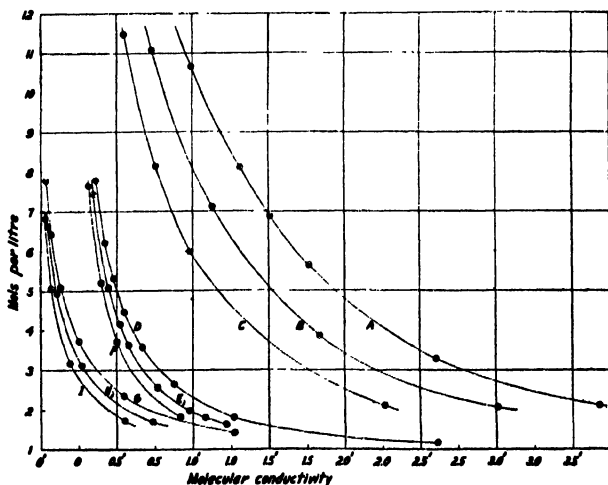


FIG. 7. Molar conductivity of formic, acetic and propionic acids in water and in aqueous hydrogen peroxide. A, D, G, above acids respectively in water; B, E, H, respectively in 10% peroxide; C, F, I, respectively in 20% peroxide.

TABLE IX
 CONDUCTANCE OF AQUEOUS SOLUTIONS OF SUCCINIC MONOPERACID

Molar concentration	0.086	0.122	0.155
Specific conductivity $\times 10^4$	2.63	3.09	3.48
Molar conductivity	3.072	2.528	2.241

 TABLE X
 CONDUCTANCE OF AQUEOUS MIXTURES OF SUCCINIC MONOPERACID AND HYDROGEN PEROXIDE

Molar conc'n of acid	Molar conc'n of peroxide	Sp. cond. $\times 10^4$	Molar cond.	Duration of experiment, hr.
0.083	0.596	2.36	2.832	39.0
0.114	0.597	2.70	2.379	15.0
0.114	1.204	2.56	2.236	21.0

totals for this sample of 75.21 cc. and of 48.74 cc. The sample was treated with a slight excess of this alkali and titrated back with 0.0206 *N* oxalic acid. The values are as follows:—alkali added, 57.50 cc.; oxalic acid added, 8.00 cc.; the difference being 49.50 cc. To another such sample were added 64.00 cc. of alkali, 14.60 cc. of oxalic acid, and the difference found was 49.40 cc. These values of 49.50 and 49.40 cc. agree well with the theoretical 48.74 cc. stated above, since some hydrolysis of the peracid would ensue on standing for even a short time with 8 to 14 cc. of excess alkali.

Thus the succinic monoperacid radical has no titratable hydrogen,—i.e., the peracid radical is un-ionized. This is in certain agreement with the conductivity results shown before.

Discussion of Results

Organic acids being weak electrolytes obey Ostwald's dilution law and their conductance is intimately related to the dielectric constant of the solvent in

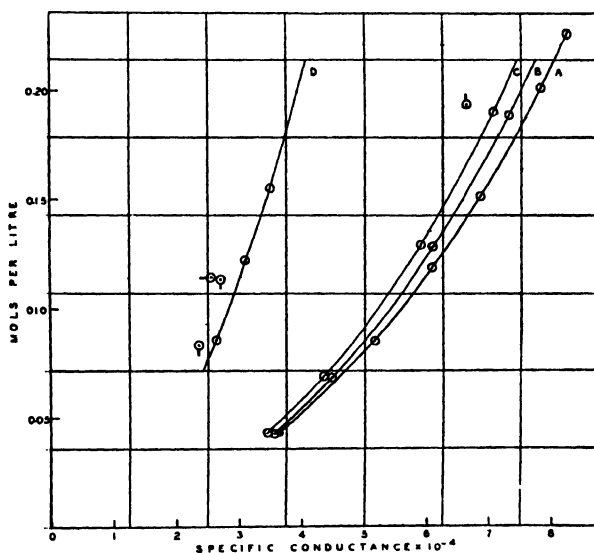


FIG. 8. Specific conductance of succinic acid and succinic monoperacid in water and in aqueous hydrogen peroxide. A, succinic in water; B, succinic in 2% peroxide; C, succinic in 4% peroxide; D, monoperacid in water. Points: \circ monoperacid in 2% peroxide; \square monoperacid in 4% peroxide; \triangle succinic in 8% peroxide.

which they are dissolved. Linton and Maass (6) having shown that the dielectric constants of water, of hydrogen peroxide and of mixtures of both are approximately the same, it might be expected that an organic acid dissolved in such a mixture would show practically the same conductivity as in water alone. Since, however, in every case a large decrease in conductivity takes place which is dependent on the concentrations of acid and peroxide, obviously the peroxide acts in such a way as to remove ions from solution. Because of the results obtained by Cuthbertson and Maass (2) with inorganic salts, the only way in which this removal could take place must be by combination with the ionogen. This would upset the equilibrium



and materially reduce the number of ions which give the specific conductivity its additive relationship. Likewise, combination of peroxide with the ionogen must produce a substance having, at least, a much lower conductivity than the acid itself.

In this connection the experiments with the very stable succinic monoperacid are of significance, for the conductivity of this compound has been shown to be almost the same as that of butyric acid; from this it becomes apparent that the action of hydrogen peroxide on the dibasic succinic acid is such as to remove from the sphere of influence one of the carboxyl groups until in the monoperacid this group is effectually eliminated. This is further enhanced by the results obtained when succinic monoperacid is dissolved in a mixture of hydrogen peroxide and water, for in this case the only existing carboxyl group is further attacked by hydrogen peroxide, tending also to its elimination as an ionizing factor.

Turning now to the monobasic acids under consideration, the fact is of importance that their instability to hydrolysis is great; so that it must be assumed, for the moment, that the acid-hydrogen peroxide complex formed on mixing has no appreciable conductivity. This assumption is justified by the results with succinic monoperacid, and must receive confirmation if the relationships of the interaction between a monobasic acid and hydrogen peroxide obey the law of mass action.

An acid HX, on ionizing gives the relationship

$$\frac{[\text{H}^+] \times [\text{X}^-]}{[\text{HX}]} = K_i, \text{ the ionization constant.}$$

This may also be expressed as

$$[\text{H}^+] \times [\text{X}^-] = K_i [\text{HX}]. \quad (1)$$

If one molecule each of acid and hydrogen peroxide unite to form a complex, we have the expression

$$\frac{[\text{HX} \cdot \text{H}_2\text{O}_2]}{[\text{HX}] \times [\text{H}_2\text{O}_2]} = K, \text{ the equilibrium constant}$$

or

$$[\text{HX} \cdot \text{H}_2\text{O}_2] = K [\text{HX}] \times [\text{H}_2\text{O}_2]. \quad (2)$$

At molar concentration formic acid is but 1% ionized, while the percentage ionization of acetic and propionic acids is about one-third of this value. Thus in these determinations it may be said that these acids exist in these concentrations almost altogether in the undissociated state. It will be seen that the specific conductivity of these acids in water is almost constant for concentrations above $2M$; should, however, much of this acid be removed by combination of the undissociated part, the number of ions carrying the current decreases, thus decreasing the specific conductivity. Therefore, at any new concentration of acid (due to removal of acid by combination with hydrogen peroxide) Equation (1) would still hold; thus

$$\frac{[H^+]_i [X^-]_i}{[HX] - [HX \cdot H_2O_2]} = K_i \quad (3)$$

This is obviously true only if the complex $HX \cdot H_2O_2$ has no appreciable conductivity of its own. But, according to Equation (2),

$$[HX \cdot H_2O_2] = K [HX] [H_2O_2];$$

therefore, Equation (3) becomes

$$\frac{[H^+]_i [X^-]_i}{[HX] - K [HX] [H_2O_2]} = K_i \quad (4)$$

or,

$$[H^+]_i [X^-]_i = K_i ([HX] - K [HX] [H_2O_2]) \quad (5)$$

In considering Equation (1), the conductivity of the ions in water may be represented by the specific conductivity; and in Equation (5) the same holds. Therefore,

$$K_i \frac{[HX]}{[HX] - K [HX] [H_2O_2]} = \frac{\text{Specific cond. of HX in } H_2O}{\text{Specific cond. of HX in } H_2O + H_2O_2},$$

or,

$$\frac{[HX]}{[HX] - K [HX] [H_2O_2]} = \frac{\text{Sp. cond. of HX in } H_2O}{\text{Sp. cond. of HX in } H_2O + H_2O_2} \quad (6)$$

In other words, the ratio of the concentration of acid in water only, to the same concentration in water and hydrogen peroxide diminished by the amount of acid which has united with the hydrogen peroxide to form an un-ionized complex, is equal to the ratio of their respective specific conductivities.

It must not be lost sight of that the relationship shown in Equation (6) holds as long as the conductivity of the hydrogen peroxide in water is negligible and the uncombined acid does not show too great a conductivity of its own in a highly concentrated solution with respect to hydrogen peroxide. The former, since hydrogen peroxide possesses a specific conductivity less than 2×10^{-6} , and the latter, except where the relative proportions of acid to hydrogen peroxide leave much acid uncombined, will not influence conductivity measurements to any appreciable extent.

TABLE XI

I	II	III	IV	V	VI	VII
Formic acid						
1.076	37.31	3.081	29.73	2.763	3.315	0.066
1.111	38.15	6.365	22.42	2.017	7.071	0.065
2.851	58.90	3.129	45.20	1.586	8.917	0.074
5.412	70.31	6.580	36.54	0.675	35.608	0.073
6.104	74.61	3.216	53.64	0.879	19.630	0.087
7.141	75.74	6.701	36.10	0.505	47.840	0.078
10.011	70.44	13.551	10.40	0.104	135.630	0.063
10.091	69.95	3.323	48.71	0.483	33.530	0.091
10.484	68.52	6.887	31.05	0.296	72.210	0.079
Acetic acid						
0.633	6.97	3.065	6.13	0.969	1.940	0.040
0.810	8.34	3.069	6.73	0.831	2.485	0.063
0.835	8.47	6.336	5.58	0.669	5.287	0.054
0.987	9.09	3.072	7.18	0.727	3.033	0.069
1.567	10.24	3.086	8.15	0.520	4.836	0.066
1.933	10.61	13.651	3.42	0.177	26.380	0.050
2.633	10.81	3.106	8.63	0.328	8.176	0.065
2.712	10.79	6.413	6.86	0.253	17.390	0.057
3.147	10.70	3.114	8.61	0.274	9.800	0.063
4.079	10.10	3.136	7.99	0.196	12.790	0.067
4.200	9.98	6.472	6.17	0.147	27.180	0.059
6.437	7.83	3.181	5.92	0.092	20.470	0.077
6.640	7.65	6.571	4.21	0.063	43.630	0.069
7.900	6.72	13.951	0.99	0.013	110.210	0.061
Propionic acid						
0.701	6.14	3.054	5.17	0.737	2.141	0.052
0.724	6.23	6.309	4.01	0.554	4.567	0.056
2.106	7.48	3.059	5.71	0.271	6.441	0.077
2.175	7.41	6.318	4.24	0.195	13.740	0.068
3.938	5.55	3.064	4.12	0.105	12.066	0.085
4.068	5.36	6.329	2.85	0.070	25.744	0.074
5.636	3.49	3.069	2.52	0.045	18.110	0.086
5.821	3.28	6.340	1.58	0.027	36.905	0.082
Succinic acid						
0.194	7.55	2.448	6.63	3.420	0.474	0.050
0.191	7.55	1.204	7.05	3.700	0.230	0.047

Table XI shows the data taken from Tables I, II, III, IV, V, VI, VII, VIII, and Figs. 2, 3, 4, 5, 6, and the vertical columns contain the following values:-

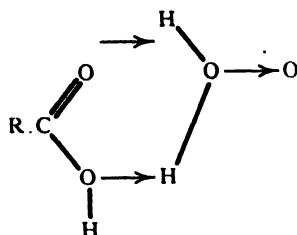
- I. Molar concentration of acid;
- II. Specific conductivity of acid $\times 10^4$ at above concentration in water only;
- III. Molar concentration of hydrogen peroxide when the concentration of acid is that given in Column I;
- IV. Specific conductivity of mixture of acid, hydrogen peroxide and water $\times 10^4$;

- V. Calculated molar conductivity of mixture;
- VI. Product of I and III;
- VII. The constant K .

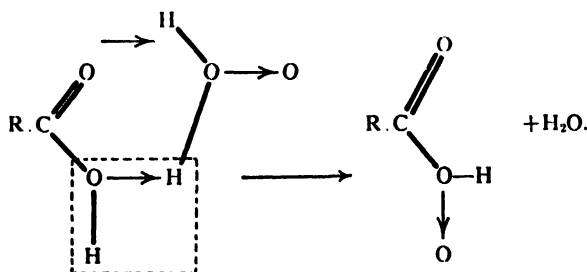
The constant K in Column VII above is calculated from Equation (6) using the values in Columns I, II, III, IV and VI.

It will be observed that these constants are well maintained except at concentrations of the monobasic acids below molar where the concentration of complex is very small. It is significant that this constant is apparently independent of the nature of the acid and its ionization constant, and affords proof of the previous assumption of interaction with un-ionized acid to form an un-ionized complex.

The constancy of conductivity decrease observed over periods of days for each mixture as recorded in Tables II, IV, VI, VIII, X, taken in conjunction with the time-growth of peracid previously studied (3, 4) permits now the formulation of the mechanism of the reaction between an organic acid and hydrogen peroxide in the initial stages before disintegration action occurs. On mixing these two reagents in water a complex is immediately formed; this consists of one molecule of acid combined with one molecule of hydrogen peroxide and is without conductivity, the hydrogen peroxide adding to the un-ionized carboxyl group of the organic acid molecule by means of co-ordinate linkages to form what Sidgwick (7) calls a six-membered chelate ring as shown—

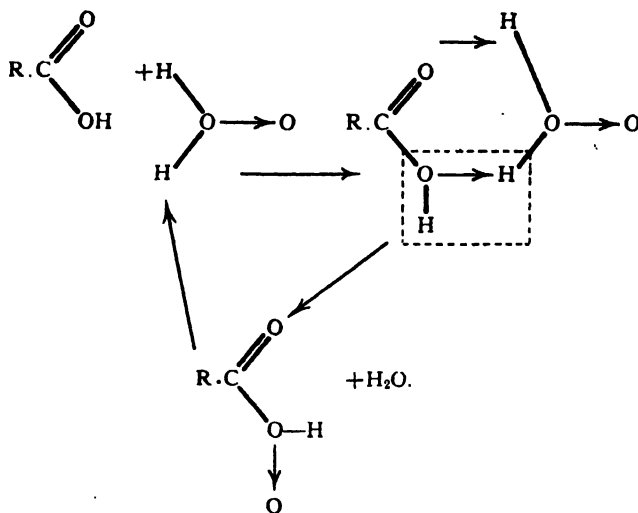


This complex may lose water slowly or rapidly, depending on its stability, to form the peracid, thus



This stability is great in the dibasic acids due to the presence of a second carboxyl group, just as the ionization of a dibasic acid is affected. Also the time required for the growth of peracid by such loss of water appears to be individually characteristic of each monobasic acid, with perhaps some relation to the ionization constant of such acid (3).

The peracid when formed hydrolyzes on addition of water to give back the original acid and hydrogen peroxide according to the scheme:



This then is the extended form of the equilibrium previously found (3, 4).

In the light of these results on conductivity change and on titration of a stable organic peracid with alkali, the generally accepted formula for an organic peracid, *viz.*, R.COOOH , is untenable. Obviously the so-called peracid is merely an oxygen addition product capable of giving up its oxygen like any highly reactive peroxide. That it is formed by means of hydrogen peroxide has been conveniently demonstrated. The formation of peracids previously reported in many cases of so-called autoxidation is therefore much simplified since in their case it is brought about by oxygen only. In no case has it been necessary to formulate any mechanism to include the primary addition of water or the primary removal of hydrogen.

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THE O-METHYLATION OF QUERCETIN¹

By J. ANSEL ANDERSON²

Abstract

The methylation of penta-acetylquercetin with dimethyl sulphate and potassium hydroxide by Freudenberg's method yields both penta- and tetramethylquercetin in the proportion of about 2:1. A modification of the method using twice the quantity of reagents yields pentamethylquercetin only (yield, 84%). There is no indication of nuclear methylation.

In the course of recent investigations it was necessary to obtain a good yield of a fully methylated derivative from a rather small quantity of tricin. Of the methods for the methylation of flavones heretofore reported, that of Freudenberg (1) appeared to be the most promising. By treating penta-acetylquercetin in boiling methyl alcohol with dimethyl sulphate and potassium hydroxide, he obtained a yield of 75—80% of fairly pure pentamethylquercetin. Gomm and Nierenstein (2) were unable to duplicate these results. The method was therefore reinvestigated.

In this laboratory the method has yielded a mixture of penta- and tetramethylquercetin in the proportion of about 2:1. The latter separates almost immediately on dilution of the reaction mixture and is filtered off. The pentamethylquercetin often remains in solution in the filtrate for several days and then separates very slowly, a property which has been previously observed by Perkin (3). In carrying out the reaction it is necessary to add the various quantities of reagents in rapid succession as directed by Freudenberg (1). If the reaction is carried out more slowly the yield of tetramethylquercetin is increased at the expense of the pentamethyl derivative.

Gomm and Nierenstein (2) report that they were unable to prepare pentamethylquercetin by this method. They obtained only tetramethylquercetin, and they suggest that Freudenberg "has apparently mistaken impure 3,7,3',4'-tetramethylquercetin for pentamethylquercetin". This is difficult to credit since the former is pale yellow whilst the latter is colorless. In view of the experience obtained in this laboratory it seems entirely probable that the method, in the hands of its originator, yielded 75—80% of almost pure pentamethylquercetin. On the other hand it appears that Gomm and Nierenstein failed to isolate this compound owing to its property of remaining in solution for long periods.

A modification of Freudenberg's method, using twice the quantity of reagents, yields 84% of fairly pure pentamethylquercetin. There are no indications of nuclear methylation. The method should have wide application for the methylation of flavones. It necessitates the preparation of the acetyl derivative, but this may be used in the crude condition and is generally obtained quite readily in good yield. Moreover, since acetylation is often used

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, in co-operation with the University of Alberta.

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in the purification of flavones of both natural and synthetic origin, the acetyl derivative may be as readily available as the flavone itself.

Experimental

Pentamethylquercetin

Penta-acetylquercetin (2 gm.) was suspended in boiling methyl alcohol (130 cc.) and treated with dimethyl sulphate (24 cc.) and aqueous potassium hydroxide (14 cc. of 50%), and then with eight further portions (6 cc.) of dimethyl sulphate and eight portions (8 cc. of 50%) of potassium hydroxide, alternately, in rapid succession. The total time taken for the addition of the reagents was about 15 min. The reaction mixture was cooled, diluted with water (300 cc.) and filtered from a trace of precipitate. After standing for two days the pentamethylquercetin, which had separated gradually in almost colorless needles, was collected, washed and dried (1.22 gm. = 84% of the theoretical), m.p. 147—148° C*. It was recrystallized three times from alcohol and obtained in colorless needles, m.p. 151—152° C. An alcoholic solution of the compound remained colorless on the addition of a few drops of alcoholic potassium hydroxide solution.

Freudenberg's Method

Penta-acetylquercetin (2 gm.) was suspended in boiling methyl alcohol (130 cc.) and treated with dimethyl sulphate (12 cc.) and aqueous potassium hydroxide (14 cc.), and then with four further portions (6 cc.) of dimethyl sulphate and four portions (8 cc. of 50%) of potassium hydroxide, alternately, in rapid succession. The total time taken for the addition of the reagents was about 10 min. The reaction mixture was cooled, diluted with water (300 cc.) and allowed to stand in the refrigerator for three hours. The pale yellow precipitate of 3,7,3',4'-tetramethylquercetin was then filtered off. The filtrate was allowed to stand in the refrigerator for one week. Pentamethylquercetin separated very slowly as a cream-colored, semi-crystalline precipitate.

The tetramethylquercetin (0.36 gm.) was recrystallized from alcohol (Norit) and obtained in pale yellow needles, m.p. 154—155° C. Four recrystallizations from alcohol raised the melting point to 159—160° C.

The pentamethylquercetin (0.76 gm.) was recrystallized from alcohol (Norit) and obtained in almost colorless needles, m.p. 144—146° C. Five further recrystallizations from alcohol yielded colorless needles, m.p. 151—152° C.

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*Melting points are not corrected.

THE YELLOW COLORING MATTER OF KHAPLI WHEAT, *TRITICUM DICOCCUM*

II. THE CONSTITUTION OF TRICETIN¹

BY J. ANSEL ANDERSON²

Abstract

Khapli wheat leaves contain a very small quantity of a trihydroxydimethoxy-flavone, tricin. Marquis yields a trace of the same compound. These two varieties also contain water-soluble coloring matters which are apparently glucosides of tricin or of a closely related compound. Dyeing tests carried out with six other wheat varieties indicate that all contain essentially the same coloring matters.

Methylation of tricin and hydrolysis of the resulting O-trimethyltricin yielded 3,4,5-trimethoxybenzoic acid and 2-hydroxy-4,6-dimethoxyacetophenone. It was therefore assumed that tricetin, the pentahydroxyflavone derived from tricin, was 5,7,3',4',5'-pentahydroxyflavone. This was synthesized from 3,4,5-trimethoxybenzoic acid and phloracetophenone by the Allan-Robinson method. Mixed melting-point determinations showed that its O-pentamethyl and O-penta-acetyl derivatives are identical with the corresponding derivatives of tricetin. The dyeing properties and color reactions of synthetic and natural tricetin are identical and are in fair agreement with those described by Badhwar, Kang and Ventkataraman (3, p. 1111) who recently reported the synthesis of the same compound.

For reasons which have been fully discussed by Newton and Anderson (12), a study is being made of the phenolic compounds of the wheat plant and of their possible role in rust resistance. A preliminary quantitative investigation of the leaves of eight wheat varieties of graded resistance to rust was reported, and it was shown that phenols were most abundant in the most rust resistant variety, Khapli. A qualitative investigation of the compounds of this class present in Khapli wheat leaves was therefore undertaken.

The main phenolic compounds of Khapli wheat leaves are yellow coloring matters of the flavone class. Of these, a water-soluble pigment, which is probably a mixture of two or more compounds, occurs most abundantly. There is also present in very small quantity, a water-insoluble flavone, tricin. The isolation of this compound was reported by Anderson and Perkin (2) and it was shown that it is a dimethyl ether of a new pentahydroxyflavone, tricetin. The constitution of the latter has now been determined as 5,7,3',4',5'-pentahydroxyflavone.

Methods for the isolation of the water-soluble dyestuff are still under investigation. The indications are that at least two compounds of this class are present. They have so far been isolated only as a semi-crystalline powder which appears to be a mixture of several compounds. On mordanted wool, this powder dyes shades closely resembling those produced by tricetin. It has the precipitation and color reactions of a flavone glucoside but does not yield a precipitate of a flavone when subjected to acid hydrolysis. Further investigation has been postponed pending the isolation of a new supply of the powder.

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Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa, Canada, in co-operation with the University of Alberta.

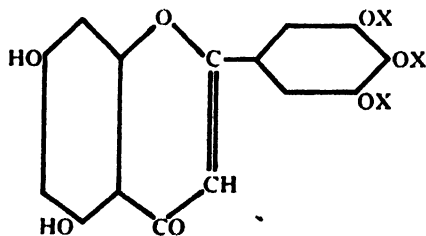
² Junior Research Biologist, National Research Laboratories, at the University of Alberta, Edmonton, Alberta.

Direct dyeing tests, using mordanted wool and ground leaves, indicate that these dyestuffs occur in all eight varieties of wheat tested, namely, Khapli, Vernal, Kanred, Kota, Kubanka, Mindum, Marquis and Little Club. The quantity of dyestuff present, as judged by the depth of color produced, varies with the variety, but is considerably greater in Khapli and Vernal, the two most rust resistant wheats. Tricin and a preparation of the water-soluble coloring matter have been isolated from both Khapli and Marquis wheats. It appears that these flavones are characteristic constituents of the wheat species.

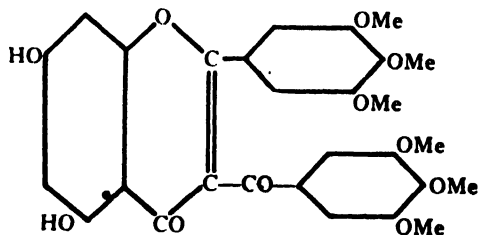
As has been reported previously (2), tricetin, by fusion with alkali, yielded phloroglucinol and an acid thought to be gallic acid. It was therefore tentatively assumed that tricetin was 5,7,3',4',5'-pentahydroxyflavone. The synthesis of this compound was undertaken by Bargellini and Monti (4) by boiling 2,4,6,3',4',5'-hexamethoxybenzoylacetophenone with hydriodic acid. The melting points of their flavone and of its acetyl derivative were respectively 60° and 24° C. lower than those of tricetin and O-penta-acetyltricetin. An attempt was made by the present author to synthesize 5,7,3',4',5'-pentahydroxyflavone by the Allan-Robinson method (1). A compound similar to, but not identical with, tricetin, was obtained in good yield. It was wrongly assumed that this was 5,7,3',4',5'-pentahydroxyflavone, and this led to the erroneous statement that tricetin had not this structure (2).

A further small quantity of tricetin was isolated recently. This was methylated and the resulting O-trimethyltricetin (O-pentamethyltricetin) was hydrolyzed in alcoholic potash. 3,4,5-Trimethoxybenzoic acid and 2-hydroxy-4,6-dimethoxyacetophenone were isolated from the reaction mixture, thus confirming the results of the previous alkaline fusion of tricetin. In these circumstances it seemed improbable that tricetin could have any other structure than that of 5,7,3',4',5'-pentahydroxyflavone. Attention was therefore redirected to the synthesis.

Further investigation showed that when phloracetophenone was heated with smaller quantities of 3,4,5-trimethoxybenzoic anhydride and sodium 3,4,5-trimethoxybenzoate, and the product hydrolyzed and isolated in the usual manner, a mixture of two compounds resulted. That produced in much smaller quantity was 5,7-dihydroxy-3',4',5'-trimethoxyflavone (I, X=Me). By methylation it yielded O-pentamethyltricetin. By demethylation it yielded tricetin, identical in all respects with the natural product. Tricetin is thus shown to be 5,7,3',4',5'-pentahydroxyflavone (I, X=H).



I



II

The compound obtained in greater quantity was identical with that obtained in the earlier synthesis. It is considered to be 3-(3'',4'',5''-trimethoxybenzoyl)-5,7-dihydroxy-3',4',5'-trimethoxyflavone (II). Analogous results have been obtained by Allan and Robinson (1) who report that the benzylation of resacetophenone yielded 7-hydroxy-3-benzoylflavone as well as 7-hydroxyflavone. Bhullar and Ventkataraman (5) find that this substitution invariably occurs in the synthesis of naphthafflavones from 2-acetyl-1-naphthol. Canter, Curd and Robertson (6) and others cited by them, find that the formation of 2-methyl-1,4-benzopyrones from o-hydroxyacetophenones by means of acetic anhydride and sodium acetate is sometimes accompanied by acetylation in the 3-position.

5,7-Dihydroxy-3',4',5'-trimethoxyflavone was prepared from the above-mentioned 3-substituted flavone by boiling it for 10 min. with 1% aqueous potassium hydroxide solution. The compound is not nearly so readily hydrolyzed in alcoholic solution. Thus, when it was boiled for two hours with 2% alcoholic potash, some of the starting product remained unattacked and at the same time most of the end-product was lost by breaking down of the pyrone ring. For these reasons, attempts to increase the yield of 5,7-dihydroxy-3',4',5'-trimethoxyflavone by more strenuous hydrolysis during the last step of the synthesis were unsuccessful. An analogous hydrolysis has been reported by Wittig, Baugert and Richter (13) who obtained 2-methylchromone by boiling 2-methyl-3-acetylchromone for one hour with 2 *N* sodium hydroxide solution.

Since the completion of this work, Badhwar, Kang and Ventkataraman (3) have published the synthesis of 5,7,3',4',5'-pentahydroxyflavone by the same method. Their results are in fair agreement with those reported in this paper, but there are certain discrepancies which are pointed out later.

Experimental

Isolation of Tricin

Khapli wheat was grown in the field until the plants were about 10 in. high, when they were cut off level with the ground. The crop was dried for several days at ordinary temperatures and finally for about 24 hr. at 35° C. The leaves were then quite brittle and were readily ground to a coarse powder.

The powder (26.5 lb.) was exhaustively extracted with 95% ethyl alcohol by percolation at room temperature. The dark green extract was concentrated by distillation *in vacuo* until the concentration of alcohol had been reduced to about 25%. The extract was filtered from a dark green sludge and worked up in portions by the following method. The solution (750 cc.) was poured into an equal volume of ether, shaken, allowed to stand, and the aqueous layer was then drawn off. The ether was washed twice with small portions of water and then extracted with dilute sodium carbonate solution (50 cc. of 2%). The pale green alkaline solution was made just acid with dilute hydrochloric acid and the dark green precipitate was filtered off and washed with chloroform. This gave crude tricetin, a pale greenish-yellow powder (total wt., 2.6 gm), which was purified by recrystallization from dilute alcohol

(Norit) and obtained in pale yellow needles (1.3 gm.) melting about 275° C.* When purified by several further recrystallizations, tricrin softens suddenly at 278° C. but does not form a clear liquid until a temperature of 288° C. is reached. Analysis: Calcd. for $C_{17}H_{14}O_7$; C, 61.8; H, 4.2; CH_3 , 9.1%. Found: C, 61.7; H, 4.4; CH_3 , 8.8%.

Marquis wheat leaves (30 lb.) yielded, by the same method, a pale yellow powder (0.06 gm.) which was purified by acetylation and identified as O-triacetyltricin by a mixed melting-point determination.

O-Trimethyltricin

O-Triacetyltricin (Anderson and Perkin, 2) (1.23 gm.) was suspended in boiling methyl alcohol (65 cc.) and treated with methyl sulphate (12 cc.) and aqueous potassium hydroxide (14 cc. of 50%), and then with eight further portions (3 cc.) of methyl sulphate and eight portions (4 cc. of 50%) of potassium hydroxide, alternately, in rapid succession. After cooling, water (125 cc.) and ether (25 cc.) were added with thorough shaking. The compound crystallized out in almost colorless needles (0.96 gm.). It was recrystallized from methyl alcohol and obtained in colorless needles, m.p. 192—193° C. Analysis: Calcd. for $C_{20}H_{20}O_7$; C, 64.5; H, 5.4; CH_3 , 20.2%. Found: C, 64.4; H, 5.5; CH_3 , 19.4%.

O-Trimethyltricin is readily soluble in methyl and ethyl alcohol. The colorless alcoholic solution shows no change on the addition of alcoholic potassium hydroxide or alcoholic ferric chloride.

Alkaline Hydrolysis of O-Trimethyltricin

O-Trimethyltricin (0.85 gm.) was refluxed for six hours in ethyl alcohol (21 cc. of 80%) with potassium hydroxide (4.25 gm.) The solution was evaporated to small volume (5 cc.) and then taken up in water (25 cc.). The solution was filtered from a little dark residue and saturated with carbon dioxide. A flocculent precipitate of the phenol separated. It was collected, washed with water and dried in a desiccator (0.40 gm.).

The filtrate was extracted several times with ether and then acidified with dilute hydrochloric acid. The acid separated at once in colorless needles which were collected and dried (0.33 gm.), m.p. 167—169° C.

The acid was recrystallized from methyl alcohol (Norit) and obtained in colorless needles, m.p. 169—170° C. The melting point was not depressed when the compound was mixed with an equal quantity of O-trimethylgallic acid melting at the same temperature.

The phenol was taken up in ethyl alcohol, filtered from a trace of insoluble inorganic residue, boiled with Norit and the solution concentrated to small volume. It separated in colorless plates melting rather indefinitely between 80° and 85° C. This melting point was not depressed when the compound was mixed with an equal quantity of 2-hydroxy-4,6-dimethoxyacetophenone, m.p. 83—85° C. The phenol was acetylated, the product was recrystallized from alcohol and was obtained in colorless needles, m.p. 105—107° C. A mixed melting-point determination showed this product to be 2-acetoxy-4,6-dimethoxyacetophenone, m.p. 106—107° C.

*Melting points in this paper are not corrected.

2-Acetoxy-4,6-dimethoxyacetophenone

Phloroglucinol was acetylated by the method of Chattaway (7). O-Tri-methylphloroglucinol was prepared from the acetyl derivative by the method of Freudenberg (8). O-Trimethylphloroglucinol was converted to 2,4,6-trimethoxyacetophenone, the latter to 2-hydroxy-4,6-dimethoxyacetophenone, and this to 2-acetoxy-4,6-dimethoxyacetophenone by the method of Kostanecki and Tambor (11).

5,7-Dihydroxy-3',4',5'-trimethoxyflavone (Tricetin 3',4',5'-Trimethyl Ether)

A mixture of phloracetophenone (Hoesch, 10) (2 gm.), 3,4,5-trimethoxybenzoic anhydride (Heap and Robinson, 9) (14.5 gm.) and sodium O-trimethylgallate (3.5 gm.) was heated in an oil-bath at 175° C. for four hours, with mechanical stirring. After cooling, the dark brown, solid mass was chipped out of the flask, ground and refluxed with alcohol. The solid did not dissolve to any appreciable extent. A solution of potassium hydroxide (4.7 gm.) in water (6 cc.) was added gradually. The solid dissolved to form a reddish-orange solution. Boiling was continued for 30 min. Most of the alcohol was then removed by distillation *in vacuo*. The residue was taken up in water (200 cc.), filtered from a trace of insoluble matter, and the filtrate was saturated with carbon dioxide. A flocculent yellow precipitate separated. This was collected, washed with water, and thoroughly pressed out. It was then triturated with warm acetic acid (20 cc.) whereupon most of it dissolved. After cooling, the pale brownish-yellow residue, *A*, was filtered off, washed with a little methyl alcohol, and dried (0.75 gm.), m.p. 265—266° C.

The acetic acid filtrate was concentrated to about one-third its volume and diluted with methyl alcohol (10 cc.). A pale yellow, crystalline precipitate, *B*, separated immediately. It was collected, washed with methyl alcohol and dried (2.57 gm.), m.p. 198—200° C.

The fraction *A* was recrystallized twice from glacial acetic acid (Norit) and obtained in pale yellow needles, m.p. 269—270° C. (Badhwar, Kang and Ventkataraman, 3, 264—265° C.). Analysis: Calcd. for $C_{18}H_{16}O_7$; C, 62.8; H, 4.7; CH_3 , 13.1%. Found: C, 62.8; H, 4.8; CH_3 , 13.0%.

5,7-Dihydroxy-3',4',5'-trimethoxyflavone is soluble in glacial acetic acid, ethyl alcohol, chloroform and ethyl acetate; more sparingly soluble in methyl alcohol, sparingly soluble in toluene, benzene and ether; and insoluble in petrol ether and water. It dissolved in concentrated sulphuric acid to give a non-fluorescent yellow solution (Badhwar *et al*, 3, yellow solution with a violet fluorescence). The almost colorless solution in hot glacial acetic acid turns bright yellow on the addition of a little sulphuric acid, and clusters of orange-yellow needles of the oxonium salt separate on cooling. The flavone dissolves in aqueous sodium hydroxide to give a bright yellow solution. In alcoholic solution it gives a reddish-brown color with a trace of dilute alcoholic ferric chloride and a dark olive-brown with more concentrated reagent.

5,7-Diacetoxy-3',4',5'-trimethoxyflavone

5,7-Dihydroxy-3',4',5'-trimethoxyflavone (0.5 gm.) was boiled with acetic anhydride (2 cc.) containing a trace of pyridine, for one hour. After cooling,

an equal volume of alcohol was added, and on standing the compound crystallized out in almost colorless needles (0.40 gm.). It was recrystallized from alcohol and obtained in clusters of almost colorless needles, m.p. 160—162° C. Analysis: Calcd. for $C_{22}H_{20}O_9$; C, 61.7; H, 4.7; CH_3 , 10.5%. Found: C, 61.5; H, 4.7; CH_3 , 10.3%.

5,7,3',4',5'-Pentamethoxyflavone (O-Trimethyltricin)

5,7-Diacetoxy-3',4',5'-trimethoxyflavone (0.5 gm.) was suspended in methyl alcohol (30 cc.) and methylated by means of methyl sulphate (18 cc.) and aqueous potassium hydroxide (23 cc. of 50%) by the method previously described. The product (0.5 gm.) was recrystallized several times from methyl alcohol and was obtained in clusters of colorless needles, m.p. 192—193° C. A mixed melting-point determination with O-trimethyltricin gave the same value. Analysis: Calcd. for $C_{20}H_{20}O_7$; C, 64.5; H, 5.4; CH_3 , 20.2%. Found: C, 64.6; H, 5.4; CH_3 , 19.6%.

5,7,3',4',5'-Pentahydroxyflavone (Tricetin)

5,7-Dihydroxy-3',4',5'-trimethoxyflavone (0.51 gm.) was suspended in a mixture of hydriodic acid (25 cc., sp. gr. 1.7) and acetic anhydride (12.5 cc.) and heated at 140° C. The compound dissolved and after a few minutes an orange-yellow precipitate began to separate. After two hours the reaction mixture was diluted with boiling water (300 cc.) and heated on the steam bath for one hour. After cooling, the pale yellow precipitate was collected, washed with water and dried (0.43 gm.). It was recrystallized from dilute alcohol and obtained in pale yellow microscopic needles, which decomposed gradually above 330° C. (Badhwar *et al.*, 3, decomposes about 310° C.). Analysis: Calcd. for $C_{15}H_{10}O_7 \cdot H_2O$; H_2O , 5.6%. Found: H_2O , 5.5%. Calcd. for $C_{15}H_{10}O_7$; C, 59.6; H, 3.3%. Found: C, 59.7; H, 3.5%.

5,7,3',4',5'-Pentahydroxyflavone is soluble in glacial acetic acid, ethyl and methyl alcohols, acetone and ethyl acetate; it is sparingly soluble in ether, very sparingly soluble in hot water and insoluble in ligroin, chloroform, benzene and toluene. It dissolves in concentrated sulphuric acid to give a non-fluorescent yellow solution (Badhwar *et al.*, 3, yellow solution with a green fluorescence). It dissolves in aqueous sodium hydroxide to give an orange-red solution. In alcoholic solution it gives a reddish-brown color with a trace of dilute alcoholic ferric chloride and a dark olive-brown with more concentrated reagent. With alcoholic lead acetate it gives a bright orange-yellow precipitate (Badhwar *et al.*, 3, brown precipitate). When an alcoholic solution of the flavone is treated with a trace of dilute alcoholic potash, a greenish-yellow color develops. On the addition of more reagent the color changes to olive-green and finally to reddish-brown. In the last stage a brown precipitate separates; this redissolves on the addition of a few drops of water to give a brownish-orange-red solution. These color reactions were compared directly with those given by tricetin and were found to be identical in all respects.

When dyed on mordanted wool, both synthetic and natural tricetin give identical colors, namely, yellow on aluminium-, lemon yellow on tin-, yellowish-

brown on chromium-, and a very dark olive (greenish-black) on iron-mordanted wool (Badhwar *et al*, 3, yellow, dull yellow, biscuit and slate grey).

5,7,3',4',5'-Penta-acetoxylavone (O-Penta-acetyltricetin)

5,7,3',4',5'-Pentahydroxyflavone (0.28 gm.) was boiled for one hour with acetic anhydride (6 cc.) containing a drop of pyridine. The product crystallized out on cooling. It was collected and again treated as previously described. The product (0.35 gm.) was recrystallized from a mixture of alcohol and acetic acid and obtained in colorless needles, m.p. 241—242° C., and this could not be raised by further recrystallization. This melting point is 2° C. lower than that previously reported for O-penta-acetyltricetin (2). However, the small sample of natural tricetin available, when acetylated as described above, yielded a derivative melting at the same temperature. A mixture of the two compounds also melted at the same temperature. Analysis: Calcd. for $C_{25}H_{20}O_{12}$; C, 58.6; H, 3.9%. Found: C, 58.7; H, 4.0%.

3-(3'',4'',5''-Trimethoxybenzoyl)-5,7-dihydroxy-3',4',5'-trimethoxyflavone

The second fraction, *B*, obtained in the synthesis was recrystallized from dilute methyl alcohol and obtained in very pale yellow needles, m.p. 203—204.5° C. Analysis: Calcd. for $C_{28}H_{26}O_{11}$; C, 62.4; H, 4.9; CH_3 , 16.8%. Found: C, 62.7; H, 5.0; CH_3 , 16.8%.

The compound is very readily soluble in glacial acetic acid, chloroform and acetone; readily soluble in ethyl alcohol, soluble in methyl alcohol, ether, ethyl acetate, benzene and toluene; very sparingly soluble in hot water and insoluble in ligroin. It dissolves in concentrated sulphuric acid to give a dark greenish-blue solution and in aqueous sodium hydroxide to produce a bright yellow solution. In alcoholic solution it gives a brownish-red color with a trace of alcoholic ferric chloride and a dark olive-brown color with more concentrated reagent.

The compound was acetylated by the method last described and recrystallized from a little acetic anhydride by the addition of alcohol. The derivative was obtained in colorless needles, m.p. 189—191° C. Analysis: Calcd. for $C_{32}H_{30}O_{13}$; C, 61.7; H, 4.9; CH_3 , 14.5; C_2H_3O , 13.8%. Found: C, 61.8; H, 4.8; CH_3 , 14.2; C_2H_3O , 14.3%.

The 3-substituted flavone (1 gm.) was boiled for 10 min. with aqueous potassium hydroxide solution (100 cc. of 1%). The solution was then cooled and saturated with carbon dioxide. The pale brownish-yellow precipitate was collected, washed, and dried (0.35 gm.). It was recrystallized from glacial acetic acid (Norit) and obtained in pale yellow needles, m.p. 269—270° C. The melting point was not depressed when the compound was mixed with an equal quantity of 5,7-dihydroxy-3',4',5'-trimethoxyflavone.

Acknowledgment

During the course of this investigation the author has been in constant correspondence with Dr. A. G. Perkin, F.R.S., Emeritus Professor of Color Chemistry and Dyeing, The University, Leeds, England, under whose personal

direction the work was begun. The author takes this opportunity of expressing his gratitude to Professor Perkin for his continued interest, kindly encouragement and most valuable advice.

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OBSERVATIONS ON THE GONADS OF MALE BIRDS AFFECTED WITH FOWL PARALYSIS (NEUROLYMPHOMATOSIS GALLINARUM)¹

BY JACOB BIELY² AND V. ELVIRA PALMER³

Abstract

Observations are presented on six male birds affected with fowl paralysis. Characteristic lymphoid infiltrations were found in the nervous system and in the testes. Lymphomatous tumors of the testes occurred in three of the birds. Evidence is presented that spermatogenesis was definitely suppressed.

In a monograph on fowl paralysis, Pappenheimer, Dunn and Cone (11) have noted that the testes of normal or paralytic fowl seemed to be, of all the viscera studied, the least favorable site for a localization of the lymphoid infiltrations that are characteristic of fowl paralysis. Furthermore, no male birds in their series showed lymphomatous tumors. Observations in this laboratory are at variance with those of Pappenheimer *et al.*

Review of Literature

While fowl paralysis occurs in both male and female birds, the number of paralyzed male birds available for post-mortem examination is comparatively small, since the majority of the males are discarded between 8 and 12 weeks of age (*i.e.*, before the usual age of onset of paralysis). Nevertheless, Pappenheimer *et al* (11) have shown that in the Leghorn flock of the Storrs Agricultural Experiment Station, the percentage incidence of fowl paralysis was about equal in both sexes.

It is interesting to note that the first account (8)* of fowl paralysis was based upon a study of four males. (Two of the four birds made a partial recovery and one died after 25 days. The fourth bird was killed four weeks after the onset of symptoms and showed the typical histopathology of fowl paralysis.)

In a series of 60 spontaneous cases of fowl paralysis examined by Pappenheimer *et al* (11) there were but four males. Three of these showed immature testes, but the exact age was not known. In the fourth case, the testes were not examined. In their experimentally inoculated chickens there were but two paralyzed males, one killed on the 122nd day, the other killed on the 137th day. The testes in these two cockerels were also immature, the seminal epithelium being almost wholly undifferentiated. On the basis of a comparison with normal cockerels of equal age, Pappenheimer concluded that in the case of the two paralyzed males, spermatogenesis was definitely delayed or suppressed. Furthermore, no lymphoid infiltrations or lymphomatous tumors were found in this series of birds.

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*See reference (11).

Various investigators have brought forward evidence that fowl paralysis may be transmitted from the adult to the chick through the egg (3, 5, 9, 12). The role of the male bird in the transmission of the disease is, however, not definitely known. Warrack and Dalling (12) state that "while we have insufficient evidence to justify a conclusion that the male bird may play a part in transmission, there are good grounds for believing that this may occur in some cases. (In 10 out of 33 outbreaks the only imported stock were cockerels.)"

Material and Methods

Out of a large number of paralyzed male birds that have been examined in this laboratory, it is proposed to deal here with six male birds showing various manifestations of the disease. The males were hatched from eggs secured at four different times from a flock in which fowl paralysis and lymphomatous tumors were prevalent. Five of the six males were crossbreds (Black Orpington ♂ × R.I. Red ♀ and Barred Rock ♀), while one male was a pure bred R.I. Red.

A careful post-mortem examination was made of each bird. Small pieces of tissue from the nervous system and viscera of each bird were fixed in Zenker's solution, embedded in paraffin and sectioned according to the usual technique. Routine histological stains were used. Only a sufficient number of sections were made to enable the authors to establish a positive microscopic diagnosis of fowl paralysis. However, serial sections of the spinal cord with the attached nerve roots and ganglia in the thoracic region were made in each case. One or both testes from each bird were examined. A summary of the histological findings on each of the six birds is included in the following notes.

Clinical Features and Pathology

W.B. 2069

Crossbred male (Black Orpington ♂ × R.I. Red ♀). Killed November 14, 1931 (128 days old).

The bird showed lack of co-ordination in walking for several days before it was killed. The left wing was droopy and spastic. The head appeared dull and pale. The bird was in fair flesh.

Autopsy. An extensive lesion was found in the left brachial plexus, extending to the corresponding segments of the spinal cord. The brachial nerves were about five times their normal size and were yellowish and translucent. The intercostal nerves on the left side were also considerably enlarged. The brain, lumbosacral plexus and sciatic nerves appeared to be normal. Scrapings from the intestinal tract contained a few coccidia. The right testis was approximately 1.4 by 0.5 by 0.5 cm., while the left was much larger, being 3.7 by 2.8 by 1.8 cm. (Fig. 5). The left testis was tumor-like, firm, grayish-yellow and translucent.

Microscopic examination. No examination was made of the gross lesions in the spinal cord and brachial plexus, since the material was used in inoculation experiments. The sciatic nerve, which did not show gross lesions, on micros-

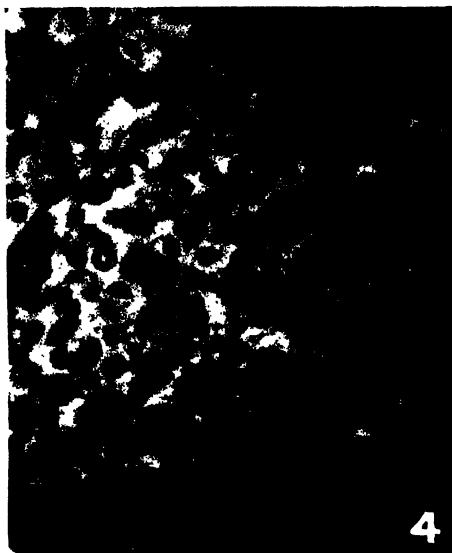
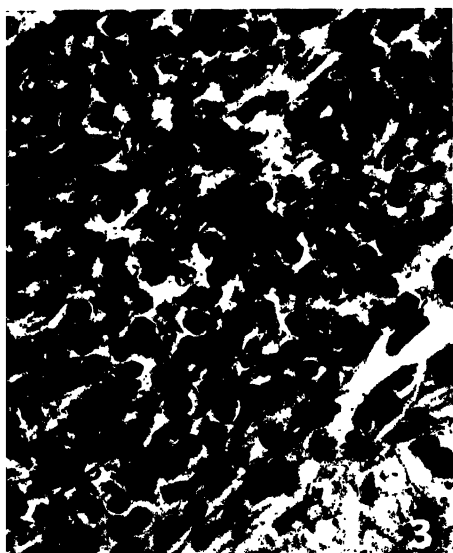
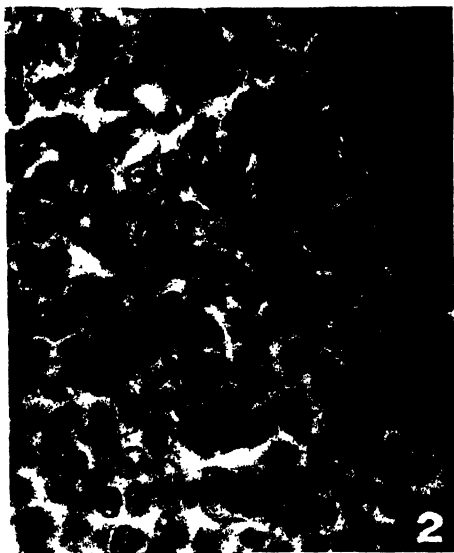


FIG. 1. W.B. 2069, male. Longitudinal section of sciatic nerve showing dense lymphoid infiltration, $\times 1200$. FIG. 2. W.B. 2069, male. Section of lymphomatous tumor of the testis. Note cells in mitosis, $\times 1200$. FIG. 3. W.B. 2814, male. Section of spinal cord showing dense lymphoid infiltration, $\times 1200$. FIG. 4. W.B. 10702, female. Section of lymphomatous tumor of the ovary. Note cells in mitosis, $\times 1200$.

copic examination revealed an extremely dense infiltration, consisting mainly of small lymphocytes, throughout the entire cross section of the nerve. Large lymphocytes, large mononuclears and plasma cells were also present. The infiltration was particularly dense around the small blood vessels. Cells showing mitosis were fairly numerous (Fig. 1).

Small dense areas of perivascular infiltration, consisting mainly of small lymphocytes and a few large ones, were seen in the liver. The spleen was normal.

Histologically the left testis had the appearance of a typical lymphomatous tumor. The seminiferous tubules were almost completely obliterated by lymphoid infiltration. Small lymphocytes predominated and were densely packed. There was also a considerable number of larger lymphoid cells with nuclei of a more vesicular character and with more cytoplasm. Cell proliferation was apparently very active, since large numbers of mitotic figures were visible (Figs. 2 and 7).

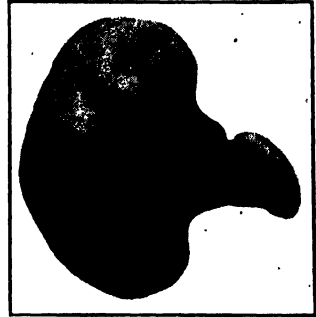


FIG. 5. W.B. 2069, male. Lymphomatous tumor of the left testis. Right testis underdeveloped. Natural size.

W.B. 2872

Crossbred male (Black Orpington ♂ × Barred Rock ♀). Killed July 15, 1931 (116 days old).

Clinical symptoms were negative. The bird appeared to be in good condition.

Autopsy. The bird was apparently normal, except that the left testis, which measured approximately 6.5 by 4 by 3 cm. was considerably larger than the right testis, which measured 1.5 by .7 by .8 cm. (Fig. 6). The left testis was very firm and grayish white in color. Scrapings from the intestinal tract showed the presence of a few coccidia.



FIG. 6. W.B. 2872, male. Lymphomatous tumor of the left testis. Right testis underdeveloped. Natural size.

Microscopic examination. No microscopic lesions were seen in sections from several blocks of the nervous system, except in the posterior root-ganglia of the thoracic region, which showed dense perivascular infiltrations composed mainly of small lymphoid cells. One or two cells in mitosis were evident.

Between the nerve fibres and the ganglion cells there was sparse infiltration with small lymphocytes. The liver showed numerous dense spherical aggregations, mainly perivascular, of lymphoid cells; mitotic figures were present. The spleen and kidneys were normal.

Histologically the structure of the left testis was very similar to that of bird No. 2069 described above. The small lymphocytes were, however, not quite as numerous as in that case. The seminiferous tubules were almost entirely replaced by lymphoid tissue. Occasionally vestiges of the tubules were seen (Fig. 8). Numerous cells showed mitosis.

W.B. 333

R.I. Red male. Killed Feb. 15, 1932 (332 days old).

The bird was fully mature and well developed. For several weeks it appeared to be dull and out of condition. Finally it developed a slight limp.

Autopsy. There was a slight thickening of the posterior root-ganglia of the spinal cord in the thoracic region. Brain, spinal cord, lumbosacral plexus and sciatic nerves appeared to be normal. In the liver there were isolated areas of round grayish white nodules. The lungs were congested and showed tiny nodules similar in appearance to those in the liver. The spleen and kidneys were normal. The intestinal tract was not examined. Both testes appeared to be normal in size and shape. One testis was slightly more grayish in color than the other.

Microscopic examination. In the cerebrum there were perivascular infiltrations around a few small blood vessels, and in the spinal cord (thoracic region) a similar infiltration was seen around small blood vessels inside the meninges. There was a very distinct and dense area of infiltration (mainly of small lymphocytes) at the junction of the roots and spinal cord. In the brachial plexus small perivascular lesions consisting of small lymphocytes, large lymphocytes and a few large mononuclears were present. Many mitotic figures were seen. Small lymphocytes, large lymphocytes, large mononuclears and a few plasma cells were scattered between the ganglion cells. The sciatic nerve appeared to be normal except for the presence of a dense perivascular infiltration in the perineurium.

The liver showed dense perivascular lymphoid infiltration. The spleen appeared normal. In the kidney there were small areas of dense perivascular lymphoid infiltration. Sparse infiltration with small lymphocytes was noted between the tubules and around the glomeruli.

The right testis was almost completely replaced by round-cell infiltration, while in the left testis there were areas of tissue with the seminiferous tubules intact (Fig. 9). Large lymphoid cells predominated. Numerous mitotic figures were present. Dense perivascular lymphoid infiltrations were seen in the tunica albuginea. The infiltration of the left testis extended from the tunica albuginea towards the centre, occupying about three-fourths of the circumference of the cross section. At the centre and at one side (except near the tunica) the seminiferous tubules showed spermatogonia, spermatocytes and spermatids. In the right testis only vestiges of tubules appeared. The small round-cell infiltrations extended into the epididymis, and some of the ducts were obliterated. In the region of some of the ductuli efferentes the infiltration was slight.

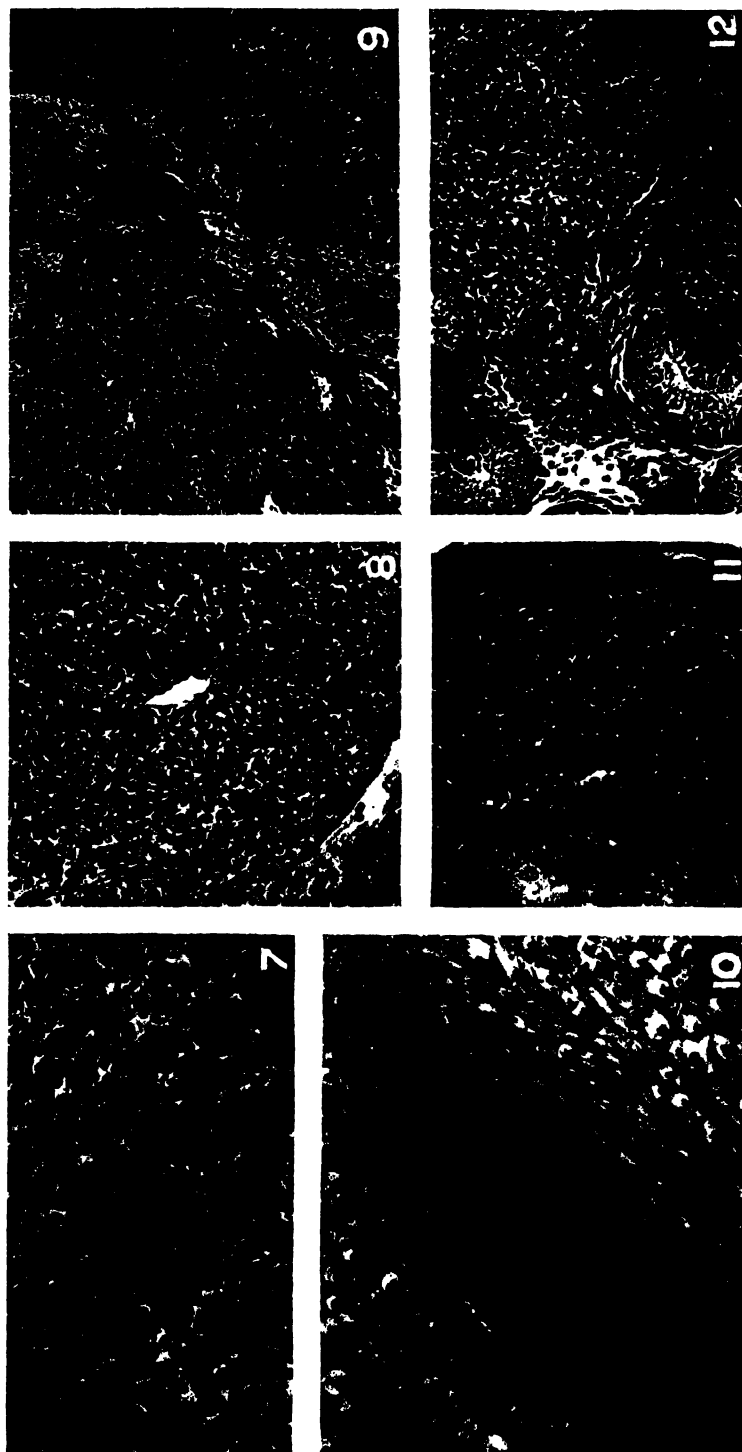


FIG. 7. W.B. 2069, male. Section of lymphomatous tumor of the testis. Note vestiges of tubules. $\times 70$. FIG. 8. W.B. 2872, male. Section of testis showing lymphoid infiltration with area of intact seminiferous tubules. $\times 350$. FIG. 9. W.B. 333, male. Section of spinal cord showing dense area of lymphoid infiltration. $\times 350$. FIG. 10. W.B. 2814, male. Section of testis showing dense area of infiltration bordering on the tunica albuginea and encroaching on the tubules. $\times 350$. FIG. 11. W.B. 311, male. Section of testis showing dense area of lymphoid infiltration. $\times 350$. FIG. 12. W.B. 2814, male. Section of testis showing dense area of lymphoid infiltration. $\times 350$.

W.B. 2814

Crossbred male (Black Orpington ♂ × Barred Rock ♀). Killed September 8, 1931 (159 days old).

This bird was thin and pale in the face, but did not show any signs of paralysis.

Autopsy. There were no gross lesions. The intestinal tract was normal. Both testes were very much undersized. The left testis was about the size of a small bean, while the right testis was half as large.

Microscopic examination. Small but dense lesions composed mainly of small lymphocytes were noted in the thoracic nerve-roots. Sparse infiltration of lymphoid cells occurred between the ganglion cells and fibres. In the white matter of the spinal cord there was seen a wedge-shaped lesion composed of an extremely dense aggregation of lymphoid cells with small lymphocytes predominating (Figs. 3 and 10).

The right testis was immature. A cross section showed several dense spherical aggregations of very closely packed lymphoid cells, with small lymphocytes predominating. The cells were less densely packed at the periphery of the lesion and penetrated between the tubules (Fig. 12). There were two small areas of dense perivascular infiltration in the tunica albuginea. The epididymis showed one small fairly dense nodule and also sparse infiltration throughout the rest of the epididymis. The left testis was composed almost entirely of connective tissue.

W.B. 308

Crossbred male (Black Orpington ♂ × R.I. Red ♀). Killed April 10, 1932 (101 days old).

This bird was normal in appearance and in good condition.

Autopsy. There was nothing abnormal except that the posterior root ganglia on both sides of the spinal cord were thickened and translucent in color. The intestinal tract was normal.

Microscopic examination. The posterior root ganglia, spinal cord and sciatic nerves showed characteristic lymphoid infiltrations. In the liver there were seen small areas of perivascular infiltration.

In the epididymis there was one large lesion composed mainly of small lymphocytes. The cells were very densely packed, and a few were in mitosis. No infiltrations were seen amongst the seminiferous tubules.

W.B. 311

Crossbred male (Black Orpington ♂ × R.I. Red ♀). Killed April 13, 1931 (103 days old).

This bird showed paralysis of the left leg April 7, 1931.

Autopsy. There were typical gross lesions of fowl paralysis in the nervous system. The intestine appeared normal. The testes were underdeveloped, but normal in shape and color.

Microscopic examination. Sciatic nerve, brachial plexus, spinal cord and liver showed massive lymphoid infiltration, which was almost of the nature of a neoplasm.

In the testes, bordering on the tunica albuginea were several lymphoid lesions, which were encroaching on the seminiferous tubules (Fig. 11). A few small, but dense, areas of small lymphocytes occurred between the seminiferous tubules towards the centre of the section. There were a few mitotic figures. Infiltration between the tubules was sparse, but there was dense perivascular infiltration in the tunica albuginea.

Discussion

The male birds described in the above summary showed gross and microscopic lesions in the nervous system that were characteristic of fowl paralysis. In addition gross and microscopic lesions were found in the testes. As already noted, the gross appearance of the testes varied considerably in the different males and in the same male. On microscopic examination the testes showed various degrees of lymphocytic infiltration, ranging from dense spherical aggregations to tumor-like masses. In fact, in the case of each of three males, one testis assumed the nature of a typical lymphomatous neoplasm. As far as the authors are aware, this is the first time that the incidence of such lesions in the testes of paralyzed birds has been recorded in the literature.

It is of importance to note that in the case of three males the neoplastic nature of one of the testes entirely suppressed spermatogenesis, while the other testis remained underdeveloped. In the remaining three males both testes appeared immature and there was evidence of lymphoid infiltration displacing and encroaching on the seminiferous tubules. The authors' data thus tend to support Pappenheimer's observation (11) that fowl paralysis may definitely delay or entirely suppress the onset of spermatogenesis. In the case of the female it has been shown by McGaughey and Downie (10) that fowl paralysis may interfere with the normal function of the ovary. They state that in one flock of birds an outbreak of fowl paralysis has strikingly arrested the maturity of young pullets and seriously interfered with the egg production of older pullets.

In discussing the natural mode of transmission of fowl paralysis, Warrack and Dalling (12) advance the hypothesis that "the hen with typical fowl paralysis ovarian tumors would seem to be bound to transmit infection to her chicks, and though absolute evidence seems wanting, it is highly probable that the male bird acts also as a transmitter." Similarly, the demonstration of typical lesions of fowl paralysis in the testes of adult male birds may be taken as presumptive evidence that the male, acting as a carrier, may transmit fowl paralysis to his progeny. But it should be noted here, that in the case of pullorum disease it has not been possible to demonstrate conclusively that *S. pullorum* can be transmitted to the chick by an infected male. Genetically, however, it has been shown that the male bird may play an important role in the transmission of resistance or susceptibility to pullorum disease (4), fowl typhoid (6, 7), and fowl paralysis (1).

On comparing the microscopic appearance of the tumor-like masses from the testes with that of similar masses from the ovary, it became evident that the tumors were essentially alike in nature. Furthermore, the lesions of both

organs were identical in appearance microscopically with the dense lymphoid infiltrations of the brachial plexus, sciatic nerve and spinal cord. This marked similarity is strikingly brought out in Figs. 1, 2, 3 and 4.

Evidence that the visceral lymphomata are a manifestation of fowl paralysis has been brought forward by several investigators (2, 11, 12). The data presented in this paper on the association of fowl paralysis with tumor-like masses in the testes would seem to strengthen further the evidence that the lymphoid infiltration in the nervous system and in the viscera are in response to a common, if not identical, etiological agent.

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CHEMICAL AND MICROBIOLOGICAL FACTORS IN SOME QUEBEC SOILS¹

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Abstract

The major soil groups in a large area of agricultural Quebec have been defined and the primary factors effecting differentiation studied. Organic-matter relations as well as the variations in mineral colloidal matter have determined the groupings adopted. Eight major soil groups are named; *vis.*, heavy clay, sandy clay, lowland podsol, upland podsol, brown earth, orchard soil, lowland muck swamp and upland muck swamp. Chemical and microbiological studies of representative virgin soils within each of the first-named six groups are reported, and field data presented.

Introduction

The object of the studies reported in this paper was to define the major soil groups in part of the province of Quebec, and to lay the foundations for a better understanding of the primary factors which effect their differentiation. Some of the soils discussed in the present paper were sampled in 1929 and analyzed during 1929 and 1930, but the greater number were collected jointly during the summer of 1930 by the Chemistry and Bacteriology Departments of Macdonald College acting in co-operation. Both microbiological and chemical data are given for the majority of the soils.

The general area from which these soils were sampled includes the lower Ottawa river valley, the Richelieu river valley and the middle St. Lawrence river valley. From the eastern boundary of Montmagny county to the western boundary of Hull county is a distance of a little more than 300 miles. This is the greatest length of the area of southern Quebec within which typical soils have been studied, its average depth being about 100 miles. For convenience, and because of the geological and climatic relations of these areas, the data presented are grouped in two ways: first, under the counties from which soils were sampled; second, soils of similar nature according to distribution from east to west within the area under consideration.

Samples of typical virgin soils were taken from the following counties, and examined: lower Ottawa river valley; Hull, Argenteuil and Vaudreuil: Richelieu river valley; Missisquoi, Iberville, Rouville and St. Hyacinthe: middle St. Lawrence river valley; Chateauguay, Huntingdon, Joliette, Yamaska, Nicolet, Lotbiniere and Montmagny. Studies of typical virgin soils from the counties of Brome, Shefford, Richmond, Sherbrooke, Stanstead and Compton have been reported elsewhere (8).

These counties include some of the most fertile agricultural land in Quebec. They also contain within their boundaries less valuable land. In Hull, Argenteuil, Joliette, Missisquoi, Lotbiniere and Montmagny counties, some

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samples, the examination of which is reported here, were taken from the higher, agriculturally less valuable lands as well as from the lower-lying soils. In Hull, Argenteuil and Joliette these upland soils are in the foothills of the Laurentian peneplain, while in Missisquoi, Lotbiniere and Montmagny they are in the Appalachian region. As will be pointed out later, the soils of the Appalachian region, of the lowland or Monteregian section, and of the Laurentian area, cannot be considered as similar. Geologically and climatically they are different, and the differences are reflected in their composition.

As the reports of the Dominion Geological Survey (4), Department of Mines, Ottawa, deal with the area described above, the geology of the counties from which samples were taken in this present investigation will not be discussed here. A discussion of the geology of a part of the Eastern Townships will be found in the bulletin referred to previously (8).

There are considerable variations in climatic conditions within this area of Quebec, and these climatic differences are reflected in the soil differences. The importance of climate in its relation to soils will be discussed and data relative to Quebec climatic conditions presented elsewhere.

This investigation has not been intended as a soil survey, nor has it been undertaken for the purpose of preparing soil maps. As stated, the object has been to group together soils of similar nature and to study their chemical and biological characteristics. In each county the district agricultural representative or someone else intimately acquainted with local soil conditions has accompanied the authors when the latter sampled the soils. In locating the most important soil areas, topographical and geological maps were consulted, and when the area of soil from which it was desired to take a type sample was selected, search commenced for virgin soil truly representative of the area. In this search the procedure followed by United States soil-survey workers was used. Normal soils were sought on smooth lands, in undulating, not flat, areas, and wherever possible at least six feet above the level of permanent water.

The method of sampling used and the treatment of samples are fully described in a previous publication (8, p. 38). The results of field examination of the soils studied are given in the succeeding pages. These data are presented by counties, not because of any soil differentiation peculiar to them, but for convenience. In the tables the subsoil samples are marked "A". Surface soils were sampled from the top 8 in.; subsoil samples were taken between depths of 16 and 24 in. Moisture and hydrogen ion concentration were determined on the fresh soil samples as soon as they reached the laboratory. Hygroscopic moisture, lime-requirement values and loss on ignition were determined on air-dried soils. All results are expressed on the moisture-free basis. The results of the five determinations mentioned in this paragraph are shown under "Field data", in Tables I to XII.

Soil Differentiation into Groups

In the previously cited bulletin (8) soil differentiation into "classes" was effected, on a basis of the organic-matter relations, into podsolized soils, brown-

TABLE I
FIELD DATA, MONTMAGNY COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygroscopic					
75 75A	60.18 24.10	3.08 3.21	5.01 5.51	3850 1820	6.10 3.33	A—4 in. medium chocolate heavy clay loam B—brownish-grey heavy clay loam to below 24 in., mottled and contains red slate bits	Sampled June 25, 1930, from permanent pasture. Original cover white pine, large elms present. Heavy clay — "terre forte" — earthworms numerous
76 76A	28.82 24.86	1.75 1.24	5.56 5.62	2300 3375	5.60 3.39	A—1 to 3 in. chocolate f.s.l.* B ₁ —4 to 7 in. dark brown f.s.l. mixed with clay patches B ₂ —10 to 14 in. brown f.s.l. C—Light grey f.s.l. mottled, to below 30 in.	Sampled June 25, 1930, from virgin soil on south bank of river 20 ft. above water level. Sugar maples and elms 2 to 4 ft. diameter present. No earthworms. "Terre franche"—sandy clay.
77 77A	66.25 50.07	11.76 12.52	5.04 5.29	8150 6750	25.78 24.85	A—6 to 12 in. reddish chocolate fine gravelly loam B—4 to 10 in. reddish fine gravelly loam—slate C—No soil below 22 in., just flat red slate fragments	Sampled June 25, 1930, from recently cut sugar maple woods. Huge stumps. Present cover young maples and balsam fir. Earthworms present. "Orchard soil"
78 78A	54.18 24.07	3.85 1.22	5.21 5.62	7370 2190	12.49 3.25	A ₁ —1 to 2 in. black raw humus A ₂ — $\frac{1}{2}$ to $\frac{1}{2}$ in. leached f.s.l. B—6 to 10 in. brown f.s.l. C—Greyish-white very f.s.l. with silt, to below 30 in. gneiss fragments.	Sampled June 25, 1930, from recently cut woodlot. Original cover, white and silver birch, spruce, balsam. Moss hummocks. "Upland podsol". No earthworms
79 79A	57.66 34.43	5.09 3.12	4.46 4.99	12690 4730	13.78 7.25	A ₁ —2 in. black raw humus A ₂ —3 to 6 in. leached f.s.l. B ₁ —4 to 6 in. red f.s.l. B ₂ —10 in. grey-brown f.s.l. C—Grey-brown f.s.l. to below 30 in.	Sampled June 26, 1930, from recently cleared land. Cover, white birch and spruce. Moss hummocks. Hardpan at 22 in. Upland podsol. No earthworms. Extreme leaching
80 80A	41.26 22.21	4.76 4.82	6.42 6.64	3410 1610	9.49 4.63	A—2 to 4 in. chocolate clay loam B—Grey-brown very heavy clay loam, non-mottled, to below 30 in., granular structure, good condition	Sampled June 26, 1930, from permanent pasture. Original cover, white pine. Present huge elms. Many earthworms. "Terre forte", heavy clay

*Fine sandy loam.

earth soils and muck-swamp soils. In the work reported in this paper, organic-matter relations are still probably the most significant factor determining the classification adopted. It has been found possible to divide the soils encountered into eight groups, most of the soils being listed as heavy clays, sandy clays, lowland podsoils and upland podsoils. The upland podsol and

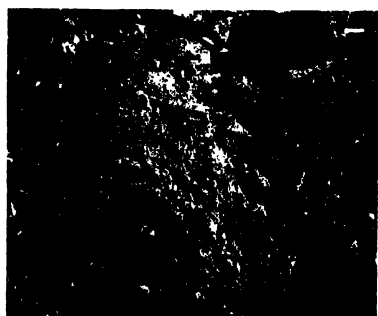
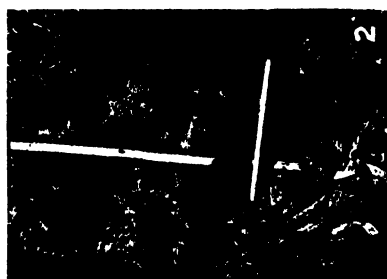


FIG. 1. Heavy clay profile. FIG. 2. Sandy clay. FIG. 3. Lowland podsol cover. FIG. 4. Lowland podsol profile. FIG. 5. Upland podsol (Appalachian) cover. FIG. 6. Upland podsol (Laurentian) profile. FIG. 7. Upland podsol (Appalachian) profile. FIG. 8. Brown earth profile.

TABLE II
FIELD DATA, LOTBINIERE COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
107 107A	54.63 22.50	2.34 0.71	4.42 5.02	7488 1248	10.03 1.68	A ₁ —3 to 5 in. black raw humus A ₂ —2 to 5 in. white leached fine sandy loam B ₁ —2 to 6 in. brown-red sand B ₂ —3 to 4 in. yellow-brown fine sandy loam C—Yellowish-white sand	Sampled Sept. 26, 1930. Woodlot, mixed forest. "Terre légère". Pine, spruce, poplar, soft and sugar maple. No earthworms. Heavily leached upland podsol
108 108A	26.87 12.68	3.12 1.59	4.75 4.70	5288 2937	8.96 3.11	A—2 to 11 in. dark brown well-decomposed organic matter B—5 to 12 in. gravelly loam C—Yellow-brown gravelly loam, rock fragments well decomposed	Sampled Sept. 26, 1930. Woodlot. "Brown earth". Elm, beech, red oak, basswood, sugar maple, canoe birch. Some earthworms. Not podsolized
109 109A	46.18 29.03	2.24 1.82	3.84 4.66	8518 2202	11.40 3.96	A ₁ —4 to 5 in. black raw humus A ₂ —1 to 6 in. white leached fine sandy loam B ₁ —2 to 4 in. yellow-brown f.s.l.* B ₂ —2 to 5 in. red-brown f.s.l. C—Yellow sand and gravel	Sampled Sept. 26, 1930. Woodlot. "Terre légère"—upland podsol. Alder, tamarack, balsam fir, white spruce, soft maple. No earthworms. Area stony and poorly drained; hardpan in B horizon in many places
110 110A	42.59 22.10	2.11 0.83	5.20 5.22	2937 1175	9.18 1.95	A ₁ —2 to 4 in. raw humus A ₂ —1 to 6 in. leached layer B ₁ —5 to 7 in. dark brown f.s.l. B ₂ —5 to 7 in. grey-brown f.s.l. C—Yellow fine sandy loam	Sampled Sept. 27, 1930. Woodlot. "Terre légère"—upland podsol. Balsam fir, white spruce, white cedar. Earthworms found. Podsolization slight in some places, heavy in others.
111 111A	39.60 27.34	3.10 2.05	5.09 5.54	3744 1761	9.08 4.48	A ₁ —2 to 4 in. brown raw humus A ₂ —2 to 3 in. leached layer f.s.l. B—7 to 11 in. yellow-brown f.s.l. C—Yellow sand overlying fragmented slate	Sampled Sept. 27, 1930. Woodlot. "Terre légère"—upland podsol. Balsam fir, white spruce, sugar maple, red birch. Some earthworms. Slate pieces in soil

*Fine sandy loam.

brown-earth soils reported here correspond to those soils reported in the previously cited reference under these names.

The soils of the area under consideration were divided into the following eight major groups on the basis of their field characteristics only:— (1) heavy clay ("terre forte"); (2) sandy clay ("terre franche"); (3) lowland podsol ("terre légère", "terre jaune", etc.); (4) orchard soil; (5) brown-earth or brown-forest soil; (6) upland podsol ("terre légère"); (7) lowland muck swamp

TABLE III
FIELD DATA, NICOLET COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
101 101A	25.91 41.14	3.33 4.46	6.14 6.86	2496 734	7.36 4.38	A—1½ to 3 in. light to dark chocolate clay loam B—4 to 14 in. mottled whitish-grey clay loam, of "nut" structure C—Grey-brown non-mottled heavy clay, underlain by blue boulder clay at 6 ft.	Sampled Sept. 3, 1930. Woodlot and permanent pasture. "Terre forte"—heavy clay. White pine stumps 3 ft. diam. Elm, spruce, white pine, balsam fir, red clover. Many earthworms.
102 102A	24.80 20.68	0.79 0.39	5.32 5.70	1615 440	4.50 0.82	A ₁ —1 to 3 in. black raw humus A ₂ —2 to 6 in. white leached f.s.l.* B ₁ —4 to 6 in. reddish f.s.l. B ₂ —4 to 6 in. red f.s.l. C—Light grey fine sand extends below 30 in.	Sampled Sept. 3, 1930. Woodlot and permanent pasture. "Terre légère"—lowland podsol. White birch, bracken, hardhack, moss-hummocks. No earthworms
103 103A	30.24 23.11	0.92 1.64	5.17 5.84	2349 734	4.19 2.48	A ₁ —1 to 3 in. dark chocolate fine sandy loam A ₂ —4 to 8 in. light grey f.s.l. with some clay and silt B—8 to 20 in. brownish-red coarse sand mixed with mottled clay C—Grey-blue heavy clay underlying	Sampled Sept. 3, 1930. Woodlot recently cleared, stumps of white pine and white birch. "Terre franche"—sandy clay. Hardhack, moss and bracken. No earthworms. Podsolization incipient
104 104A	19.89 18.70	1.09 1.17	5.32 5.75	2496 1174	3.85 2.44	A ₁ —1 to 3 in. black raw humus A ₂ —3 to 12 in. grey-white leached coarse sand B ₁ —10 to 14 in. dark red coarse sand B ₂ —1 to 4 in. blackish-red true hardpan "ortstein" C—Yellow-grey coarse sand	Sampled Sept. 3, 1930. Woodlot recently cleared of white pine, white birch and poplar. "Terre légère". Bracken, club moss, sphagnum moss. No earthworms. Heavily leached lowland podsol. Some clay at depth
105 105A	14.25 11.08	2.17 1.25	5.38 5.51	2936 881	5.59 1.95	A ₁ —1 to 2 in. light chocolate v.f.s.l. A ₂ —2 to 3 in. light yellow v.f. sand with some patches of silt and clay B—8 to 10 in. mottled whitish-grey v.f. sand, some clay C—At least 40 ft. of fine sand, silt and clay	Sampled Sept. 3, 1930. Woodlot "Terre jaune". White pine, white birch. Present cover; white cedar, white pine, and white birch. No earthworms. Resembles no other soil sampled in the area studied
106 106A	37.88 24.07	4.43 2.65	5.64 5.98	3377 3903	11.69 3.08	A—4 to 8 in. medium chocolate clay loam B—4 to 30 in. hard-packed coarse sand and clay C—Grey-brown heavy clay	Sampled Sept. 4, 1930. Woodlot, original deciduous forest. "Terre forte"—heavy clay. Ash, ironwood, maple, basswood, yellow birch. Earthworms found

*Fine sandy loam.

TABLE IV
FIELD DATA, YAMASKA COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
87 87A	31.89 23.76	1.64 5.25	4.10 5.35	4774 661	6.37 1.00	A ₁ —2 to 4 in. black raw humus A ₂ — $\frac{1}{2}$ in. black f.s.l. A ₃ —3 to 6 in. heavily leached f.s.l. B ₁ —4 to 8 in. red-brown v.f.s.l. (ortstein) B ₂ —4 to 8 in. mottled v.f.s.l.* C—Light grey v.f.s.l., extends below 30 in.	Sampled July 23, 1930. Woodlot. "Terre légère" very heavily leached low-land podsol. Small white pine, balsam and white birch. Ferns and moss-hummocks prevalent. No earthworms found. Representative of a large area
88 88A	28.45 33.05	4.67 5.61	5.86 5.53	2423 955	8.59 4.20	A—1 to 2 in. light chocolate clay loam B—Grey-brown non-mottled heavy clay loam extends below 30 in. This horizon is 8 to 20 ft. deep.	Sampled July 23, 1930. Permanent pasture. "Terre forte"—heavy clay. White pine stumps 4 ft. diam. Elm, poplar, birch, red raspberry, bracken. Earthworms numerous
89 89A	96.30 43.06	5.35 4.43	4.66 6.32	7121 734	16.99 3.64	A ₁ —4 to 6 in. black raw humus A ₂ —4 to 8 in. white-yellow to brown-red v.f. sand mixed with clay B—4 to 6 in. mottled brown-grey heavy clay C—Light grey very heavy clay extends below 30 in.	Sampled July 23, 1930. Woodlot. "Terre franche"—sandy clay. Large white pine stumps. Mixed red spruce, poplar, beech, white birch. Poorly drained except on knolls. No earthworms
90 90A	18.43 15.42	2.05 5.09	4.80 6.34	2790 507	6.25 2.49	A ₁ —3 to 5 in. medium chocolate f.s.l. A ₂ —3 to 4 in. coarse white sand A ₃ —5 to 6 in. yellow-grey v.f.s.l. B—Yellow to grey-brown hard-packed clay, extends below 30 in.	Sampled July 23, 1930. Woodlot and permanent pasture. White pine stumps. "Terre franche"—sandy clay. White pine, white birch, soft maple, poplar. Level land, well drained. Some earthworms found
91 91A	24.13 25.79	1.76 2.70	5.25 6.40	2202 374	5.05 2.32	A ₁ —2 to 4 in. black f.s.l. A ₂ —2 to 6 in. grey-white f.s.l. B—6 to 10 in. mottled grey-brown clay C—Mottled grey-brown clay to below 30 in.	Sampled July 24, 1930. Permanent pasture. "Terre franche"—sandy clay. White pine stumps 3 ft. diam. Balsam fir, hardhack, swamp grass. No earthworms found
92 92A	22.85 33.16	3.06 4.15	6.60 4.25	2202 661	8.17 3.92	A ₁ —3 to 5 in. light chocolate loam A ₂ —2 to 4 in. grey-white clay loam B—Light brown-grey non-mottled heavy clay loam C—Blue glacial boulder clay at 8 to 10 ft.	Sampled July 24, 1930. Recently cleared white pine land. "Terre forte"—heavy clay. Elm, white pine 2 ft. diam. Earthworms numerous

*Very fine sandy loam.

TABLE V
FIELD DATA, JOLIETTE COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
93 93A	44.39 17.59	1.88 0.53	4.76 5.03	3906 734	6.60 0.92	A ₁ —2 to 4 in. raw humus A ₂ —2 to 6 in. dark grey ash-color; leached layer, sandy loam B ₁ —2 to 6 in. rusty red to dark brown f.s.l. B ₂ —10 to 14 in. brownish-red f.s.l.* C—Yellowish-grey f.s.l.	Sampled Aug. 26, 1930. Woodlot and permanent pasture. "Terre légère". Balsam fir, white birch, club moss, sphagnum moss. Poorly drained lowland podsol. No earthworms
94 94A	31.24 8.54	3.71 1.24	7.26 7.98	734 none	12.94 3.72	A—6 to 8 in. dark chocolate sandy loam B—4 to 12 in. medium-brown friable gravelly loam with many limestone fragments C—Light greyish-white f.s.l., underlain by rock	Sampled Aug. 26, 1930. Woodlot. "Brown earth". Large sugar-maple and elm. Bedrock of limestone in many places at about 2ft. Numerous large earthworms Soil in good physical state
95 95A	28.47 31.17	1.43 2.90	5.35 6.22	2423 807	4.83 2.56	A—2 to 4 in. dark chocolate clay loam B—6 to 8 in. light bluish-grey clay with patches of sand C—Brownish-grey mottled clay loam	Sampled Aug. 26, 1930. Woodlot, cut 10 years, pasture. "Terre franche"—sandy clay. White pine stumps, 2 ft. diam. Elm, fir, Canada thistle. Earthworms found
96 96A	55.28 29.82	4.25 3.63	4.88 5.86	5947 734	19.48 2.80	A—4 to 6 in. light chocolate clay loam B—6 to 8 in. whitish v. fine sand with clay, very hard packed C—Brownish- to bluish-grey non-mottled light sandy clay loam	Sampled Aug. 26, 1930. Woodlot. "Terre franche"—sandy clay. Mixed forest. White pine, poplar, silver birch, soft maple, some white birch. Earthworms found

*Fine sandy loam.

("terre noire"); (8) upland muck swamp. Studies of Groups 1-6 are reported in this paper but Nos. 7 and 8 are not dealt with as only mineral soils are discussed; the black muck soils, in which organic matter is completely dominant, present problems of their own.

The heavy clays, sandy clays and lowland podsols, as grouped here, are to be found throughout the valleys of the Ottawa, Richelieu and St. Lawrence rivers. Strata of glacial boulder clay underlie the sandy clays and lowland podsols at varying depths. Sometimes the areas of exposed clay cover several square miles, but are usually less than half-a-dozen square miles in extent. After the great beds of clay were deposited from the waters of the Champlain sea, rivers issuing from the melting ice-front carried down the now superimposed sand and gravel deposits. The whole lowland country is thus inter-

TABLE VI
FIELD DATA, JOLIETTE COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
97 97A	45.35 29.59	4.30 3.23	5.56 5.37	4111 3083	11.25 6.01	A ₁ —1 to 3 in. dark chocolate fine sandy loam A ₂ —Commonly absent, but when present about ½ in. deep leached f.s.l.* B—5 to 24 in. brownish-red fine sandy loam C—Yellowish f.s.l.	Sampled Aug. 26, 1930. Permanent pasture. Laur-entian "upland podsol". White pine stumps, up to 4 and 5 ft. diam. Balsam fir, poplar, white birch, sugar maple, some flat club-moss. Earthworms found. Podsolization incipient
98 98A	29.55 27.28	3.84 3.08	5.77 6.24	1982 374	10.47 2.08	A—1 to 2 in. dark chocolate clay loam B—4 to 12 in. greyish-white compacted f.s.l., with clay C—Greyish-brown slightly mottled clay loam	Sampled Aug. 26, 1930. Woodlot, cleared 5 years. "Terre franche". White pine stumps 2 ft. diam. Sumach, red raspberry among the stumps. Earthworms found. Typical tobacco soil of Joliette county
99 99A	23.35 10.02	3.52 1.71	6.14 7.45	2056 none	11.36 3.93	A—4 to 6 in. dark chocolate clay loam B—14 to 16 in. light brown silty clay loam, very friable	Sampled Aug. 27, 1930. Woodlot and permanent pasture. "Brown earth". Elm, ash, linden, sugar maple. Earthworms found. Limestone bedrock at 20 in. Soil in good physical state, but "drouthy"
100 100A	20.72 16.58	0.93 0.52	4.61 5.25	2863 734	4.25 1.04	A ₁ —1 to 2 in. raw humus A ₂ —5 to 8 in. leached f.s.l. B ₁ —4 to 6 in. dark brownish-red f.s.l. B ₂ —14 to 20 in. mottled brown f.s.l. C—Yellowish f.s.l.	Sampled Aug. 27, 1930. Woodlot and permanent pasture. "Terre légère". Black spruce, hemlock, white birch 2 ft. diam. at 3 ft. above ground level. No earthworms. A heavily leached lowland podsol soil

*Fine sandy loam.

woven with strips and areas of heavy clay, sandy clay and sand or gravel, the latter being most heavily leached.

The orchard soils occur generally at the base of the Monteregian hills or at the foot of some slope where organic matter and mineral fragments are thoroughly mixed. The effect of each component, organic or mineral, is to make the elemental constituents of the other component more available through chemical and biological action. Where orchard soils occur at heights greater than about 400 ft. above mean sea level, it is because of a coincidence of the occurrence to a considerable depth in the soil of much organic matter mixed

TABLE VII
FIELD DATA, ST. HYACINTHE COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
62 62A	49.60 31.10	3.50 2.70	5.34 5.74	4050 1580	9.59 2.50	A—7 in. very dark grey, almost black clay loam B—8 in. grey-brown coarse sand mixed with clay C—Very heavy blue clay, mottled, to below 24 in.	Sampled June 10, 1930, from woodlot. Original cover; elm and maple: present cover; linden, elm, beech, maple. "Terre franche"—sandy clay
63 63A	37.00 38.30	2.10 3.90	4.85 7.24	4700 950	6.49 3.33	A ₁ —3 to 4 in. black, decomposed pine needles and debris A ₂ —2 to 6 in. grey-white clayey sand B ₁ —4 in. red mottled clay B ₂ —Brown mottled clay to 24 in.	Sampled June 10, 1930, from woodlot. Original cover, white pine. Present cover; white pine, hard and soft maple. "Terre franche"—sandy clay
64 64A	37.00 26.80	1.20 1.50	4.97 6.88	3415 675	5.06 1.88	A ₁ —2 to 4 in. dark brown f.s.l. A ₂ — $\frac{1}{2}$ to 4 in. leached f.s.l. B ₁ —Yellow-brown f.s.l. B ₂ —Heavy brown-grey clay, mottled, to below 24 in.	Sampled June 10, 1930, from woodlot. Original cover, white pine. Present cover; soft maple, elder, white birch, tamarack. Lowland podsol. "Terre légère"
65 65A	35.70 21.10	2.50 1.10	6.06 6.68	3200 1590	5.50 1.40	A ₁ — $\frac{1}{2}$ in. f.s.l. (raw humus) A ₂ —2 in. leached f.s.l.* B ₁ — $\frac{1}{2}$ to 3 in. red f.s.l. with ortstein lumps B ₂ —2 to 7 in. red-brown f.s.l. B ₃ —Brown-grey clay, mottled, mixed with sand, to 24 in.	Sampled June 11, 1930, from permanent pasture. Original cover, white pine. Present cover; willow, club-moss, hardhack, blueberries. Lowland podsol. "Terre légère"
66 66A	43.20 32.30	3.40 4.80	5.19 6.13	5100 1590	7.38 3.81	A—4 to 5 in. chocolate clay loam B—4 in. clay, unmottled C ₁ —16 in. friable grey clay loam C ₂ —Heavy blue clay at 24 in. slightly mottled	Sampled June 11, 1930, from woodlot just cut over. Original cover, white pine. Huge pine stumps. Heavy clay—"Terre forte"
67 67A	28.10 24.90	1.00 1.10	6.13 6.68	6225 1350	2.81 1.14	A ₁ —4 in. light chocolate f.s.l. A ₂ —3 to 6 in. grey f.s.l. B ₁ — $\frac{1}{2}$ to 1 in. reddish f.s.l. B ₂ —Light brown f.s.l. extends below 24 in.	Sampled June 11, 1930, from woodlot just cleared. Original cover, white pine. White birch trees present. Sandy clay — "Terre franche"—slight leaching

*Fine sandy loam.

with rock fragments, the component minerals of which are of great agricultural value.

The brown earths are quite closely related to the orchard soils, but they do not possess the fertility nor the abundance of well-decomposed organic matter characteristic of the latter.

TABLE VIII
FIELD DATA, MISSISQUOI COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
70 70A	31.91 41.17	3.40 1.88	6.38 6.37	3620 2260	11.23 5.34	A—5 to 8 in. dark chocolate silty clay loam B—11 to 19 in. grey-brown to reddish silty clay loam C—Yellow-grey silty clay to below 24 in.	Sampled June 18, 1930, from woodlot and permanent pasture. "Brown earth". Limestone ridges. Cover, sugar maples and white cedar trees. Earthworms present
71 71A	39.80 49.90	3.44 1.72	6.45 6.59	3080 1380	9.13 3.99	A—2 to 4 in. dark chocolate sandy loam B—3 to 8 in. reddish-brown f.s.l.* C—Easily crumbled, high iron sandstone ledge	Sampled June 18, 1930, from permanent pasture. Huge sugar maples, elms and apple trees. Red soil. Numerous earthworms. "Orchard soil"
72 72A	37.49 44.95	2.81 1.23	5.15 5.25	4050 1880	8.39 2.89	A—2 to 8 in. chocolate brown f.s.l. B—7 to 17 in. brown f.s.l. C—Greyish silt and very fine sand to below 24 in. Water-rounded pebbles in C	Sampled June 18, 1930, from permanent pasture. Original cover deciduous trees, maple, elm, ash, poplar. "Brown earth". No earthworms
73 73A	40.41 47.21	3.05 1.06	5.45 5.61	5890 2100	10.11 2.64	A ₁ —2 to 4 in. black raw humus A ₂ — $\frac{1}{2}$ to 2 in. leached f.s.l. B ₁ —2 to 8 in. reddish f.s.l. B ₂ —6 to 16 in. tight-packed and almost impervious grey fine sand, silt and clay C—Loose grey f.s.l. below 24 in.	Sampled June 19, 1930, from permanent pasture. Original cover white pine. Present cover; soft maple, hemlock, moss hummocks and hardhack. "Terre légère"—lowland podsol. Many pebbles in subsoil
74 74A	31.10 28.52	2.84 3.77	6.05 6.92	2000 2050	7.51 3.73	A—2 to 4 in. chocolate f.s.l. B ₁ —5 in. grey to reddish f.s.l. B ₂ —10 in. mixed fine sand and grey-brown clay C—Blue clay extends from 16 in. deep to below 24 in.	Sampled June 19, 1930, from woodlot and permanent pasture. Original cover, white pine. Huge stumps. Many earthworms. "Terre franche"—sandy clay. Podsolization incipient

*Fine sandy loam.

The typical upland podsoils occurring throughout the Appalachian hills and foothills of southeastern Quebec are quite unlike those of the Laurentians in chemical composition. By far the greater area of the Eastern Townships is covered with these soils. Lotbinière, Arthabaska, Drummond, Shefford, Brome, and the eastern part of Missisquoi counties form a rough border line, to the south and east of which will be found soils answering in the main to the description of the upland podsoils of the Appalachian region. Upland muck-swamp soils and some brown earths will also be found in this upland region.

TABLE IX
FIELD DATA, SOILS FROM VARIOUS COUNTIES

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygros-copic					
Chateauguay County							
11 11A	35.22 27.40	4.93 5.43	5.65 5.91	4115 2450	11.36 4.86	A—9 in. chocolate brown clay loam B—Sticky, grey-blue clay loam, mottled, extends below 24 in.	Sampled July 8, 1929, from permanent pasture and woodlot. Huge elm trees. Earthworms numerous. "Terre forte"—heavy clay
14 14A	31.12 19.76	3.08 1.77	5.78 6.58	3075 1000	7.82 2.14	A—4 to 6 in. chocolate clay loam B—Medium clay loam, mottled, with sand patches, to below 24 in.	Sampled July 9, 1929, from woodlot and permanent pasture. Original cover:— white pine: present; elm, linden, soft maple. "Terre franche"—sandy clay. Earthworms numerous
Huntingdon County							
18 18A	35.13 19.36	3.95 2.28	6.50 6.96	2200 1080	13.17 2.65	A—1½ to 8 in. chocolate clay loam B—Friable whitish clay with whitish sand patches, mottled, to below 24 in.	Sampled July 9, 1929, from woodlot just cleared. Huge elm trees present. "Terre franche"—sandy clay. Many earthworms
Iberville County							
68 68A	39.72 46.53	7.05 5.70	5.49 5.50	6100 5480	23.41 10.76	A—6 in. chocolate brown gravelly loam (syenite fragments) B—Light brown sandy to gravelly loam to below 36 in.	Sampled June 16, 1930, from maple woods. Huge maple trees. Numerous earthworms. "Orchard soil"
Rouville County							
69 69A	37.80 48.90	6.33 3.51	6.45 6.25	2040 2600	23.30 9.05	A—4 to 8 in. dark chocolate gravelly loam (syenite fragments) B—Dark reddish-brown gravelly loam to below 24 in.	Sampled June 17, 1930, from sugar maple woods. Huge sugar maple trees. Numerous earthworms. "Orchard soil"

The existence in New York State of mineral soils very high in organic matter and low in lime, namely, the Volusia soils, has been pointed out by Bizzell (1). In these respects the Volusia soils resemble the upland podsol soils of the area under consideration.

To enable effective comparisons to be made between soils listed under the various groups, Tables XIII to XXIII have been prepared showing their chemical and biological variations. In these tables the soils in each group

TABLE X
FIELD DATA, VAUDREUIL COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygroscopic					
81 81A	45.18 35.10	6.90 5.18	5.46 5.81	5450 3040	13.44 5.03	A—3 to 6 in. dark chocolate heavy clay loam B—4 to 12 in. reddish clay with red sandstone fragments C—Heavy clay, grey-blue, mottled, to below 24 in.	Sampled July 8, 1930, from woodlot. American elms present 4 ft. diameter and huge sugar maples. Stones and boulders. Heavy clay—"terre rouge". Earthworms
82 82A	38.93 22.82	3.58 3.21	6.97 6.75	2100 1280	8.01 4.06	A—1 to 3 in. chocolate clay loam B—18 to 20 in. light grey sandy clay loam C—Almost white sandy and clay loam to below 30 in., well-drained	Sampled July 8, 1930, from permanent pasture. White pine stumps present 4 ft. diameter. Present cover, white pine and American elms. "Terre blanche" — sandy clay. Earthworms
83 83A	54.25 35.39	10.26 7.42	5.30 5.14	11300 9150	31.60 23.71	A ₀ —1 in. maple leaves and debris A ₁ —5 to 8 in. dark, friable, gravelly loam B—Reddish-brown gravelly loam, to below 24 in.	Sampled July 8, 1930, from woodlot. Sugar maples of 2 ft. trunk diameter or more. Apple trees thrive in this soil. "Orchard soil"
84 84A	32.91 41.64	4.32 5.84	7.25 7.65	1200 850	9.95 4.25	A ₁ —3 to 6 in. chocolate clay loam A ₂ —3 to 6 in. yellow clay loam B—8 to 12 in. grey brown clay C—Reddish brown heavy clay loam to below 30 in.	Sampled July 9, 1930, from permanent pasture. Original cover white pine, huge elms present. Red sandstone fragments in B and C horizons. Earthworms numerous
85 85A	38.93 36.33	5.70 6.07	5.76 6.45	4100 1350	10.25 4.72	A—3 to 6 in. chocolate clay loam B—8 to 10 in. orange to grey heavy clay loam C—Grey brown heavy clay loam to below 30 in.	Sampled July 9, 1930, from recently cleared wood lot. "Terre forte"—heavy clay. Original cover, white pine. Elms and willows present. Earthworms present
86 86A	55.01 28.40	2.88 2.70	4.75 6.36	4280 1840	8.67 2.77	A ₁ —2 in. black raw humus f.s.l.* A ₂ — $\frac{1}{2}$ in. black f.s.l. A ₃ —2 to 4 in. leached f.s.l. B ₁ —4 to 8 in. red f.s.l. (ortstein) B ₂ —6 to 10 in. brown f.s.l. (mottled) C—Light grey very f.s.l. with blue clay	Sampled July 9, 1930, from woodlot. Original cover hemlock and balsam fir of 18 in. trunk diameter. "Terre légère"—lowland podsol. No earthworms present

*Fine sandy loam.

are listed according to their distribution from east to west. The values in the tables are all expressed on the basis of moisture-free soil.

TABLE XI
FIELD DATA, ARGENTEUIL COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygroscopic					
34 34A	13.52 14.25	3.48 3.36	5.30 5.60	4190 3150	8.68 4.63	A ₁ — $\frac{1}{2}$ in. light chocolate f.s.l.* A ₂ —2 to 4 in. leached f.s.l. B ₁ —2 in. dark red f.s.l. (ortstein) B ₂ —16 in. light brown f.s.l. C—Hard-packed grey to yellow-white f.s.l. with some clay, to below 24 in.	Sampled July 28, 1929, from permanent pasture. No earthworms. Original cover deciduous trees. Moss hummocks, hardhack, sorrel prevalent. Very badly leached lowland podsol. Stony,
35 35A	10.76 22.29	3.27 6.56	4.78 4.94	4275 1390	6.17 4.47	A ₁ —1 to 2 in. dark chocolate f.s.l. A ₂ —3 to 4 in. white, extremely heavy clay loam B—Extremely heavy, sticky, grey-brown clay loam	Sampled July 28, 1929, from newly cleared woodlot. Original cover, tamarack trees. Heavy clay. Strikingly white clay layer. No earthworms
36 36A	19.14 22.06	9.57 6.94	5.18 5.49	5650 1340	17.36 4.12	A—4 to 5 in. dark chocolate clay loam B—Medium brown clay loam, mottled red-brown, extends below 24 in.	Sampled July 28, 1929, from permanent pasture. Earthworms present. White pine original cover, huge stumps present. Red clover thrives here. Heavy clay
37 37A	9.16 14.16	3.41 2.52	5.11 6.10	56 2260	9.04 3.52	A ₁ — $\frac{1}{2}$ to 5 in. light chocolate loam A ₂ —0 to 4 in. leached f.s.l. B ₁ —0 to 3 in. dark red f.s.l. B ₂ —15 to 18 in. brown f.s.l. C—Yellow-brown fine sand and gravel, some clay.	Sampled July 28, 1929, from long-cleared permanent pasture. Hemlock original cover. Lowland podsol, in places. Many pieces of red, soft sandstone present. No earthworms
38 38A	16.50 10.84	4.33 2.94	5.55 6.05	5650 3450	10.43 5.24	A ₁ —1 to 3 in. light chocolate f.s.l. A ₂ —0 to 1 in. leached f.s.l. B ₁ —0 to 2 in. dark red f.s.l. B ₂ —Light brown f.s.l., extends below 24 in.	Sampled July 29, 1929, from long-cleared permanent pasture. No earthworms. Sugar maples, original cover. Laurentian upland podsol. Moss hummocks present
39 39A	28.67 18.63	4.98 4.04	5.61 6.04	4000 1650	13.56 2.68	A—1 to 3 in. very dark chocolate heavy clay loam B—12 in. bluish to dark grey very heavy clay loam C—Grey brown heavy clay	Sampled July 29, 1929, from land just cleared. Earthworms present. Original cover coniferous trees. Heavy clay. Clovers thrive on this soil

*Fine sandy loam.

Analytical Methods

All the methods used in the chemical analyses have been described previously (8). They are in most cases the methods of the American Association of Official Agricultural Chemists.

In the microbiological studies the following methods have been used:—

TABLE XII
FIELD DATA, HULL COUNTY SOILS

Sample No.	Moisture, %		pH	Lime requirement, lb. CaO per acre	Loss on ignition, %	Description of soil horizons	Remarks
	In fresh sample	Hygroscopic					
40 40A	34.53 18.49	9.52 5.27	6.01 7.10	3790 1200	17.99 3.43	A—3 in. dark chocolate clay loam B—3 in. blue-grey friable clay loam C—Very heavy, sticky, grey-blue, mottled clay, to below 24 in.	Sampled August 6, 1929, from woodlot. Original cover standing, chiefly American elm and butter-nut trees. Heavy clay. Earthworms present
41 41A	23.11 18.72	4.88 4.17	6.19 6.23	3250 1900	9.26 4.71	A—4 to 5 in. light chocolate heavy clay loam B—Heavy blue-grey, friable clay loam to below 24 in.	Sampled August 6, 1929, from woodlot. Original cover standing, elm, white pine, balsam fir and cedar. Heavy clay. Earthworms present
42 42A	20.25 19.73	6.69 5.45	6.86 7.90	2225 730	13.39 3.04	A—4 to 6 in. medium chocolate clay loam B—4 in. whitish, friable clay loam C—Grey-blue, friable clay loam to below 24 in.	Sampled August 6, 1929, from long-cleared, unploughed land. Original cover white pine and American elm. Heavy clay. Earthworms present
43 43A	8.75 8.53	3.52 2.41	5.61 5.85	4050 2000	9.06 3.01	A—2 to 3 in. very light chocolate fine sandy loam B—12 to 15 in. brown f.s.l.* C—Light grey, hard-packed f.s.l. with clay patches	Sampled August 7, 1929, from long-cleared, rough and stony permanent pasture. Original cover, sugar maple and American elm. "Brown earth". No earthworms
44 44A	19.08 15.90	6.91 4.53	5.63 5.35	6350 2150	18.84 3.32	A—3 to 4 in. light chocolate clay loam B—3 to 4 in. hard-packed, friable, whitish clay loam C—Grey-white, tight clay loam	Sampled August 7, 1929, from woodlot in permanent pasture. Original cover standing, white pine and American elm. Sandy clay. No earthworms
45 45A	10.41 12.76	3.74 3.66	5.70 6.41	2860 1660	6.21 2.47	A— $\frac{1}{2}$ to 1 in. medium chocolate clay loam B—8 to 10 in. whitish clay with sand patches C—Grey-brown clay loam with brown sand patches, to below 24 in.	Sampled August 7, 1929, from long-cleared permanent pasture. Original cover standing, white pine, American elm and white ash. Sandy clay. No earthworms

*Fine sandy loam.

Water-holding Capacity

(This is the maximum water content held against gravity (7)). Air-dried soil, (10 to 20 gm.), was ground in a porcelain mortar and after passing through a 1-mm. mesh sieve, was placed in a small funnel, having at the neck a small cone of filter paper, saturated with distilled water. The soil was made to settle by tapping the funnel, and distilled water run in slowly from a burette

TABLE XIII

PERCENTAGES OF SOME CONSTITUENTS OF HEAVY CLAY SURFACE SOILS AND SUBSOILS

Sample number	Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₂	Ratio C/N	Ratio SiO ₂
										Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂
80	23.42	2.23	2.84	2.62	3.55	0.31	0.22	0.16	11.5	2.48
80A	25.15	2.50	3.22	2.99	0.55	0.07	0.16	0.08	8.2	2.25
75	18.48	2.32	2.28	1.90	2.17	0.19	0.20	0.12	11.7	3.39
75A	24.11	2.15	1.99	2.21	0.56	0.06	0.21	0.08	9.5	2.65
106	24.89	1.67	3.07	2.25	4.44	0.44	0.28	0.16	10.1	2.18
106A	23.58	2.26	2.50	2.30	0.68	0.04	0.11	0.08	18.4	2.71
101	23.05	2.10	2.58	1.98	2.62	0.16	0.14	0.10	16.6	2.60
101A	27.16	2.23	3.62	2.54	0.82	0.05	0.12	0.08	16.1	2.08
88	23.72	2.50	3.10	2.60	3.26	0.19	0.15	0.12	17.1	2.47
88A	27.07	2.52	4.05	2.68	0.38	0.05	0.13	0.06	7.3	2.11
92	20.43	2.11	2.54	1.92	3.36	0.23	0.17	0.12	14.5	3.04
92A	26.63	2.14	3.64	2.95	0.66	0.05	0.11	0.07	12.2	2.20
66	20.66	2.21	2.77	2.08	3.00	0.18	0.13	0.10	16.4	3.01
66A	24.28	2.83	3.47	2.65	0.43	0.05	0.12	0.07	8.8	2.45
11	21.63	1.73	1.75	1.69	4.67	0.39	0.12	0.36	11.9	3.03
11A	28.20	1.52	2.68	1.84	0.31	0.06	0.05	0.22	5.7	2.29
81	22.59	1.43	2.19	3.32	5.84	0.43	0.26	0.21	13.7	2.49
81A	25.36	1.85	2.27	2.91	1.58	0.08	0.18	0.08	19.8	2.47
85	24.74	1.95	2.13	2.48	3.54	0.26	0.14	0.12	13.4	2.32
85A	26.75	2.74	3.08	2.40	0.31	0.05	0.14	0.07	6.9	1.78
84	20.02	2.77	2.57	2.45	4.21	0.33	0.21	0.16	12.7	2.97
84A	26.31	2.66	3.52	2.74	0.19	0.05	0.14	0.06	4.0	2.19
35	21.10	2.25	1.85	1.93	1.85	0.11	0.17	0.05	16.2	3.12
35A	28.60	2.49	3.24	1.44	0.36	0.23	0.10	0.03	15.9	2.03
36	24.16	1.62	1.73	1.45	7.82	0.39	0.31	0.12	19.9	2.14
36A	28.28	2.02	3.45	2.48	0.37	0.03	0.21	0.02	10.6	2.02
39	19.40	2.50	1.54	3.12	7.25	0.27	0.29	0.15	26.8	3.07
39A	25.23	2.97	1.32	3.18	0.37	0.01	0.32	0.03	28.1	2.50
42	25.56	2.94	3.86	2.21	6.25	0.36	0.34	0.13	17.5	1.96
42A	30.09	3.30	4.52	2.92	0.17	0.04	0.19	0.09	5.0	1.85
41	27.87	4.70	3.73	2.60	3.98	0.29	0.28	0.12	13.5	1.97
41A	28.49	4.82	4.03	2.51	1.19	0.11	0.26	0.05	10.7	2.02
40	27.67	2.86	2.87	2.32	7.83	0.50	0.32	0.22	15.6	1.66
40A	27.60	3.93	3.07	2.24	0.47	0.03	0.20	0.09	18.5	2.13

until the first drop in excess of water-holding capacity exuded from below the cone. The value was taken from the mean of two or three determinations.

TABLE XIV
PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF HEAVY CLAY SURFACE SOILS

Sam- ple num- ber	Date collected	Period incu- bation, days	Water- holding cap'y, fresh soil, %	Moisture in fresh soil as % of water- holding cap'y	Moisture in soil after in- cubation as % of water- holding cap'y	Nitrate nitrogen in fresh soil, parts per million	Nitrate nitrogen after incu- bation, parts per million	Carbon dioxide, mgm. per 100 gm. soil per 100 hr.	Date plated	Bacteria and actino- myces per gm. soil
80	June 25	449	59.0	61.9	49.1	trace	228.4	25.3	June 28	7,098,000
75	June 25	435	43.0	131.8	35.5	0.00	82.5	19.9	June 28	2,673,000
106	Sept. 4	487	60.8	65.9	49.1	2.47	85.9	25.9	Sept. 8	5,996,000
101	Sept. 3	456	45.5	49.7	33.4	4.00	109.0	11.0	Sept. 8	15,680,000
88	July 23	449	64.2	37.1	28.4	0.00	92.8	37.2	Aug. 1	29,250,000
92	July 24	448	53.0	37.4	23.0	trace	94.6	37.4	Aug. 1	17,960,000
66	June 10	399	52.7	75.5	49.3	0.00	116.8	37.3	June 14	2,675,000
81	July 8	462	60.4	64.0	54.9	2.00	210.8	41.6	July 10	14,210,000
85	July 9	463	56.6	58.0	52.0	1.96	252.5	22.3	July 10	5,454,000
84	July 9	463	55.3	41.1	41.3	0.00	242.3	28.1	July 10	20,090,000

Counts of Bacteria and Actinomyces

Fresh moist soil (10 to 20 gm.) was plated on Thornton's count medium (10), four or five plates being prepared, usually at a dilution of 1:100,000.

Nitrate Nitrogen

Harper's modification (6) of the phenol-disulphonic acid method was used.

Carbon Dioxide

This was determined from 400 gm. of dried soil remoistened to 60% of saturation. The carbon dioxide was collected from six soils at one period in an 18-tube apparatus based on that described by Fred and Waksman (3), the gas being collected from each soil sample and absorbed in three tubes each containing 33.3 ec. of standard barium hydroxide. Residual barium hydroxide was titrated in the proximal tube at intervals, and in the three tubes at the end of a period of from 14 to 19 days. A check on the apparatus showed that during a similar period the amount of carbon dioxide passing through an empty flask was equivalent to 0.90 gm. per 100 hr.; this amount has been subtracted to give the amounts shown in the tables.

The temperature of the room in which the soils were placed for this test varied during the period of the work (July, 1931, to January, 1932). The averages of the highest and lowest ranges of temperatures were 24.9° and 19.9° C.

Counts of Fungi

These were made in the Plant Pathology Department, Macdonald College, according to the directions in Fred and Waksman's laboratory manual (3, p. 98).

Discussion of Soil Groups

Heavy Clays (terre forte)

Soils grouped as heavy clays are those in which, in all the horizons investigated, mineral colloidal matter is distinctly dominant.

TABLE XV

PERCENTAGES OF SOME CONSTITUENTS OF SANDY CLAY SURFACE SOILS AND SUBSOILS

Sample number	FeO ₂ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₂	Ratio C/N	Ratio SiO ₂ FeO ₂ + Al ₂ O ₃ + TiO ₂
76	17.32	1.30	0.69	1.60	2.33	0.19	0.18	0.11	12.2	4.07
76A	18.31	1.96	0.86	1.59	0.77	0.08	0.14	0.07	10.0	3.93
103	16.63	1.91	0.86	1.79	2.37	0.10	0.10	0.10	24.2	4.49
103A	22.17	2.01	1.76	2.20	0.62	0.05	0.20	0.08	12.2	3.04
89	17.80	1.84	1.86	1.61	7.74	0.39	0.14	0.19	20.0	1.03
89A	25.89	2.71	3.19	2.44	0.31	0.03	0.12	0.10	9.4	2.37
90	17.78	1.39	1.34	1.96	2.65	0.19	0.12	0.11	13.8	3.82
90A	24.39	2.60	1.79	2.68	0.89	0.04	0.18	0.08	22.3	2.78
91	18.96	1.25	1.39	1.41	2.15	0.13	0.11	0.10	16.1	3.78
91A	23.10	2.26	2.38	2.13	0.78	0.03	0.15	0.06	27.1	2.79
67	17.17	2.89	1.06	1.69	1.36	0.09	0.11	0.06	15.1	4.21
67A	17.72	3.58	1.84	2.14	0.33	0.05	0.19	0.06	6.4	4.00
63	17.31	2.31	1.18	1.82	3.24	0.17	0.08	0.09	19.3	3.90
63A	24.34	3.01	2.76	2.15	1.29	0.05	0.15	0.06	23.9	2.54
62	17.04	2.51	1.83	2.00	3.94	0.31	0.23	0.14	12.6	3.62
62A	21.97	3.18	2.42	2.07	0.59	0.04	0.15	0.07	14.4	3.02
74	13.92	1.68	1.45	2.65	2.77	0.23	0.10	0.12	12.3	4.89
74A	23.69	1.95	2.59	3.00	0.41	0.06	0.17	0.06	6.7	2.64
96	17.70	1.59	1.11	2.30	9.51	0.45	0.12	0.22	22.5	3.10
96A	25.07	2.36	2.43	2.98	0.82	0.03	0.23	0.08	21.2	2.45
95	19.07	2.73	1.45	2.75	2.43	0.13	0.16	0.10	10.8	3.39
95A	20.50	2.94	2.73	2.34	0.63	0.03	0.18	0.07	19.0	2.55
98	22.36	1.84	1.91	2.86	4.51	0.20	0.31	0.13	21.7	2.59
98A	24.22	2.63	2.37	3.28	0.62	0.03	0.29	0.08	22.9	2.50
14	21.63	1.73	1.75	1.69	2.98	0.24	0.12	0.28	11.9	3.03
14A	28.20	1.52	2.68	1.84	0.25	0.03	0.02	0.32	5.7	2.29
18	22.02	2.52	2.35	2.04	5.86	0.38	0.17	0.42	12.3	3.08
18A	22.85	2.80	2.08	2.10	0.38	0.04	0.05	0.27	8.8	2.95
82	27.82	2.86	3.06	2.47	2.97	0.26	0.19	0.13	11.2	2.08
82A	24.04	3.01	3.09	2.12	0.78	0.08	0.14	0.08	9.9	2.52
45	20.97	3.26	3.03	2.46	2.16	0.20	0.29	0.12	10.7	2.54
45A	27.86	3.39	3.32	2.94	0.36	0.03	0.24	0.07	10.6	2.27
44	24.30	3.57	2.97	2.37	9.30	0.37	0.37	0.17	25.0	2.36
44A	27.34	3.58	4.11	2.79	0.40	0.04	0.21	0.01	9.7	2.13

The heavy clays of Montmagny county on the upper gulf of St. Lawrence show surprisingly small fundamental differences from those of Argenteuil and Hull counties on the lower Ottawa river. These boulder clays are made of

TABLE XVI

PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF SANDY CLAY SURFACE SOILS

Sample number	Date collected	Period incubation, days	Water-holding cap'y, fresh soil, %	Moisture in fresh soil as % of water-holding cap'y	Moisture in soil after incubation as % of water-holding cap'y	Nitrate nitrogen in fresh soil, parts per million	Nitrate nitrogen after incubation, parts per million	Carbon dioxide, mgm. per 100 gm. soil per 100 hr.	Date plated	Bacteria and actinomyces per gm. soil
76	June 25	449	50.9	51.3	39.5	trace	114.3	12.9	June 28	3,475,000
103	Sept. 3	483	40.1	73.1	59.3	trace	109.1	3.4	Sept. 8	7,127,000
89	July 23	449	66.8	136.2	97.1	0.00	181.2	35.9	Aug. 1	12,030,000
90	July 23	449	36.7	51.6	36.9	0.00	63.5	25.6	Aug. 1	12,131,000
91	July 24	448	33.6	66.7	63.7	0.00	112.1	19.6	Aug. 1	6,343,000
67	June 10	399	32.9	82.4	44.8	0.00	78.7	20.3	June 14	3,689,000
63	June 10	400	44.4	78.6	47.5	0.00	78.7	28.4	June 13	1,577,000
62	June 10	400	50.0	92.2	79.5	0.00	134.2	66.8	June 13	3,203,000
74	June 19	441	43.5	65.1	44.6	0.00	117.2	17.0	June 20	4,273,000
96	Aug. 26	464	65.0	78.5	53.4	trace	188.7	17.1	Aug. 28	5,030,000
95	Aug. 26	464	42.2	64.0	52.4	trace	188.6	5.6	Aug. 28	6,473,000
98	Aug. 26	464	43.8	58.7	45.3	trace	153.5	8.2	Aug. 28	21,290,000
82	July 8	464	53.9	65.7	20.5	0.00	93.8	46.3	July 10	13,290,000

"rock flour" which must have settled from colloidal suspension in water rather evenly over wide areas during long periods of time. It is probable that the turbid waters of the Champlain sea possessed a considerable degree of homogeneity in the distribution of this colloiddally suspended rock flour during the ages while sedimentation and deposition of the clays was occurring.

Climate is known to affect profoundly the nature of soils, but it is probable that our climatic variations show their smallest effect on the heavy clays. Up to the time of the loss of a considerable amount of their cementing bases through leaching, these clays show the least response to climatic variations of any of the groups under study. What seems to occur more commonly in the case of the heavy clays is the washing away and erosion of the entire surface soil mass, rather than the excessive selective leaching of certain chemical constituents. There is consequently less disproportion between the acidic and basic groups of soil constituents than is evident in soils of less colloiddality. In fact, excellent balance between their basic and acidic constituents is characteristic of this group of soils.

Another important characteristic of these soils is their relatively high content of the oxides of iron, aluminium and titanium. These oxides form the cement which preserves soil structure; they hold the silica and other major soil constituents together and, when present in quantity, they make the soil a coherent system rather than a number of systems in disconnected layers.

However, even in the heavy clays, the subsoils are distinctly richer in the sesquioxide bases than the surface soils. In all the soil groups studied there is a large and significant difference between the surface soils and the subsoils in this respect. Thus, while the heavy clays have suffered the least from

TABLE XVII

PERCENTAGES OF SOME CONSTITUENTS OF LOWLAND PODSOL (HEAVILY LEACHED)
SURFACE SOILS AND SUBSOILS

Sample number	Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₂	Ratio C/N	Ratio SiO ₂
										Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂
104	15.59	1.66	0.77	1.95	2.11	0.09	0.08	0.09	24.0	4.68
104A	19.76	2.24	1.29	1.80	1.06	0.03	0.12	0.06	31.2	3.53
102	15.24	2.31	0.94	1.82	3.09	0.12	0.12	0.11	26.2	4.81
102A	17.93	2.86	1.60	1.84	0.58	0.02	0.21	0.06	25.2	4.10
87	9.00	0.48	0.63	1.17	3.46	0.13	0.03	0.09	27.0	8.96
87A	15.23	1.79	0.86	1.64	0.28	0.03	0.08	0.07	11.2	5.32
73	14.35	1.05	0.71	1.59	4.45	0.27	0.07	0.11	16.7	4.91
73A	14.00	1.50	0.86	1.52	0.95	0.05	0.05	0.07	18.2	5.42
64	15.68	2.10	0.92	2.06	2.75	0.16	0.06	0.10	17.3	4.47
64A	18.87	2.99	2.04	1.75	0.34	0.04	0.14	0.07	8.3	3.60
65	16.87	2.38	0.91	1.83	2.25	0.12	0.05	0.09	19.6	4.11
65A	19.12	3.24	1.87	1.64	0.25	0.03	0.17	0.06	7.4	3.60
93	15.67	1.57	0.90	1.93	3.70	0.17	0.06	0.12	12.2	4.54
93A	16.74	2.25	1.26	1.66	0.87	0.02	0.09	0.09	21.3	5.41
100	17.05	2.18	0.69	2.29	3.69	0.09	0.06	0.07	41.0	4.10
100A	19.85	3.08	1.30	2.21	0.60	0.02	0.24	0.06	31.6	3.51
86	16.37	1.92	1.14	1.53	3.48	0.19	0.13	0.13	18.1	4.36
86A	22.58	2.58	2.45	2.08	0.27	0.03	0.15	0.06	7.9	2.90
34	16.52	1.59	0.68	1.54	3.07	0.24	0.17	0.12	12.9	4.35
34A	27.08	2.01	1.50	2.52	1.28	0.02	0.27	0.07	64.8	2.47
37	19.03	1.82	1.47	2.44	4.12	0.12	0.11	0.12	33.8	3.40
37A	20.80	2.21	2.07	2.34	0.87	0.07	0.20	0.09	12.5	3.27

leaching, they also are being changed by the unceasing effects of climatic conditions.

Although decidedly well supplied with organic carbon and with nitrogen, the heavy clay soils are in addition the best supplied of any of the soil groups with calcium and magnesium. They possess also relatively high percentages of total potassium and phosphorus.

One observable difference in soils within this group is the higher phosphorus content of those from the lower Ottawa valley over those from the St. Lawrence and Richelieu valleys. In general, also, the heavy clays in the western part of the area under consideration have higher percentages of the sesquioxide bases than those of the eastern part. Examination of additional samples from these areas is desirable in order to check these conclusions.

Sandy Clays (terre franche)

This group includes those soils which, within a few inches of the surface, contain sand mixed quite uniformly with clay, in thin sheets or layers rather

TABLE XVIII
PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF LOWLAND PODSOL SOILS

Sam- ple num- ber	Date collected 1930	Period incu- bation, days	Water- holding cap'y, fresh soil, %	Moisture in fresh soil as % of water- holding cap'y	Moisture in soil after in- cubation as % of water- holding cap'y	Nitrate nitrogen in fresh soil, parts per million	Nitrate nitrogen after in- cubation, parts per million	Carbon dioxide, mgm. per 100 gm. soil per 100 hr.	Date plated	Bacteria and actino- myces per gm. soil
104	Sept. 3	488	26.7	70.4	40.6	trace	86.7	4.3	Sept. 8	4,414,000
102	Sept. 3	488	30.2	79.5	65.4	2.92	129.5	4.6	Sept. 8	5,707,000
87	July 23	449	44.2	68.6	56.0	0.00	71.0	15.5	Aug. 1	2,354,000
73	June 19	441	52.6	71.1	57.2	0.00	126.4	24.6	June 20	4,215,000
64	June 11	400	39.2	91.3	70.3	0.00	111.0	29.3	June 13	1,835,000
65	June 11	399	35.4	93.8	70.1	0.00	80.2	21.7	June 13	1,771,000
93	Aug. 26	446	44.4	95.7	95.2	trace	112.3	17.0	Aug. 28	4,192,000
86	July 9	463	46.6	111.8	94.7	0.00	114.2	23.8	July 10	2,469,000

than in pockets. In many cases they show incipient podsolization but in no case is a distinct leached layer apparent, but rather only a slight variation in shade between horizons. Though rather closely related to the lowland podsol group, the sandy clays are distinguishable from them in the virgin state by their physical appearance, as well as by chemical differences.

It will be noted by reference to Tables XIII and XV that the sandy clays fall little short of the heavy clays in the balance of their fertility constituents and in the total percentages. In all cases observed, however, the surface soils of the sandy clays differ more markedly from the subsoils, than in the case of the heavy clays.

It is a significant fact that the sandy clays and the lowland podsol soils are more poorly drained than the heavy clays. This is caused by accumulation in the lower layers of clay particles carried down from the fairly open layers of the surface. This accumulated dispersed clay in the subsoil prevents the free passage of water, resulting in a water-logged soil, with all its disadvantages. When one considers the characteristic flat, or very gently sloping, topography of the clay regions of this province, together with the clogged condition of the subsoil referred to above, the significance of poor drainage as a factor causing low fertility in these soils may be appreciated. As a general rule the subsoils of the heavy clays are in much better physical condition than those of the sandy clays.

This transportation of clay particles from upper to lower layers is directly caused by the acid organic matter present in the surface layer. If the concentration of lime is sufficient to ensure coagulation of the organic soils to gels, the organic matter acts altogether beneficially. If lime is deficient, and its deficiency in the surface soils of the sandy clays is indicated in no uncertain manner by their high lime-requirement values, the soluble organic matter of the surface soils directly causes the loss of clay from these surface soils. Both the organic matter and the mineral clay particles are colloidal in nature. The

TABLE XIX

PERCENTAGES OF SOME CONSTITUENTS OF "ORCHARD" SURFACE SOILS AND SUBSOILS

Sample number	Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₂	Per cent detritus above 1 mm. diameter (air-dry basis)	Ratio C/N	Ratio SiO ₂ Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂
77	20.57	1.71	1.51	1.57	10.25	0.75	0.51	0.33	55.06	13.8	2.05
77A	28.96	1.54	1.46	1.75	7.54	0.47	0.61	0.28	73.68	16.0	1.45
71	22.72	0.39	1.29	4.09	4.02	0.34	0.29	0.16	21.67	11.8	2.57
71A	21.82	0.49	1.59	3.92	0.84	0.09	0.25	0.07	22.49	9.4	3.01
69	18.33	1.38	1.97	1.98	9.97	0.77	0.32	0.32	59.60	12.9	3.00
69A	20.49	1.39	2.32	1.59	4.07	0.29	0.24	0.16	68.81	14.1	2.99
68	17.68	1.37	1.48	1.79	9.78	0.71	0.20	0.28	53.78	13.8	3.03
68A	24.54	1.95	2.78	1.47	4.07	0.30	0.18	0.16	77.43	13.7	2.21
83	17.81	2.34	1.46	2.43	19.44	0.97	0.32	0.33	56.25	20.0	2.48
83A	22.06	1.80	1.19	2.58	9.50	0.58	0.34	0.23	57.20	16.5	2.17

acid organic matter in colloidal solution protects the clay particles, keeping them in suspension, and preventing their precipitation until the lower levels of the soil are reached.

The total content of lime in the sandy clays is about the same as in the heavy clays, but the high lime requirement of the surface soils as compared with that of the subsoils illustrates the acid condition in the surface layers of these soils, the acidity being produced from organic matter in the absence of active calcium. *Lowland Podsoils (terre légère)*

As previously mentioned, these soils are related to the sandy clay soils, the main difference between the two being that the lowland podsoils have distinct leached layers and have less clay in their surface soils, although they usually possess subsoils which may be grouped only as clays.

The lowland podsol soils, in that they have suffered extremely from leaching, and because of the fact that in the virgin state they contain the least amount of organic matter of any of the soil groups studied, are the most typical podsol soils, as defined by Russian workers, thus far encountered in this province. The distinctions between this group of heavily podsolized soils and the "upland podsol" group will be discussed later.

In most cases the lowland podsol soils occur on water-deposited sand or gravel beds. As in the case of the two previously discussed soil groups, they occur anywhere throughout the lowland region (below 400 ft. above sea level) of the area under consideration.

The surface layers of these lowland podsol soils are strikingly deficient in sesquioxide bases, phosphorus and available calcium, though the phosphorus content of the subsoils is by no means low, and the total calcium content as a whole is high. The magnesium, carbon and nitrogen contents of the soils as a

TABLE XX

PERCENTAGES OF SOME CONSTITUENTS OF "BROWN EARTH" SURFACE SOILS AND SUBSOILS,
AND OF AN UNCLASSIFIED SOIL

Sample number	Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₃	Ratio C/N	Ratio SiO ₂ / Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂
Brown earth soils										
108	19.10	0.97	1.11	1.48	3.43	0.26	0.12	0.18	13.4	3.39
108A	16.27	1.06	1.33	1.71	1.09	0.08	0.12	0.11	14.3	4.43
72	16.74	0.97	0.85	2.11	3.55	0.28	0.13	0.13	12.5	3.94
72A	17.13	1.02	1.10	2.23	0.78	0.07	0.14	0.06	10.5	4.24
70	19.00	0.83	1.39	1.53	4.60	0.40	0.18	0.16	11.5	3.24
70A	21.85	0.82	2.28	1.71	1.14	0.13	0.16	0.08	8.8	2.97
94	14.61	2.83	1.23	1.84	5.66	0.47	0.17	0.19	43.5	4.47
94A	16.23	2.91	1.58	1.58	1.23	0.11	0.16	0.11	12.0	4.41
99	17.20	2.62	1.65	1.82	4.91	0.32	0.22	0.21	23.0	3.77
99A	19.47	3.73	2.74	2.38	1.21	0.10	0.23	0.11	15.2	3.38
43	20.94	3.13	2.09	2.00	4.24	0.37	0.23	0.16	11.3	2.94
43A	25.12	3.59	2.71	2.03	0.60	0.03	0.21	0.05	17.7	2.50
Unclassified soil										
105	19.37	1.74	1.24	1.93	2.35	0.14	0.25	0.11	17.1	3.50
105A	20.37	1.72	1.59	1.93	0.81	0.04	0.18	0.07	23.1	3.45

whole are the lowest of any of the groups studied. This low content of magnesium is interesting and probably highly significant. The values of the ratio MgO/CaO in the various soil classes illustrate the condition of these soils with respect to magnesium. It is clear that these lowland podsol soils present a set of problems for study different from the Appalachian upland podsol group to be discussed later.

Orchard Soils

The most fertile soils encountered in these studies belong to this group, and are arbitrarily named "orchard" soils because: (a) apple trees, even without spraying or any other attention, have been observed to thrive on them; (b) they are unsuitable for use as general agricultural soils due to their stony nature; and (c) they possess certain characteristics which clearly mark them off from the most closely related group, the "brown earths". They have been observed, generally at a height about 300 to 400 ft. above present mean sea level, and virgin soils of the class have been sampled and analyzed from Missisquoi, Iberville, Rouville, Vaudreuil and Montmagny counties. They have been observed in other regions also but the virgin soils were not sampled.

These orchard soils are excellently drained and the organic matter, even to considerable depth, is mixed with flat-sided and angular rock fragments as

TABLE XXI

PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF "ORCHARD", BROWN EARTH AND UNCLASSIFIED SURFACE SOILS

Sam- ple num- ber	Date collected	Period incu- bation, days	Water- holding cap'y, fresh soil, %	Moisture in fresh soil as % of water- holding cap'y	Moisture in soil after in- cubation as % of water- holding cap'y	Nitrate nitrogen in fresh soil, parts per million	Nitrate nitrogen after in- cubation, parts per million	Carbon dioxide, mgm. per 100 gm. soil per 100 hr.	Date plated	Bacteria and actino- myces per gm. soil
Orchard soils										
77	June 25	449	77.1	70.7	60.7	trace	261.5	36.8	June 28	3,904,000
71	June 18	442	54.8	66.3	55.7	trace	141.4	27.4	June 20	3,463,000
83	July 8	464	84.6	52.0	43.4	0.00	496.4	61.8	July 10	8,784,000
Brown earth soils										
108	Sept. 26	465	39.2	60.7	45.6	0.00	78.1	26.6	Oct. 6	6,977,000
72	June 18	442	56.8	61.1	45.9	0.00	102.5	20.4	June 20	3,671,000
70	June 18	442	53.4	53.4	49.7	trace	162.5	29.1	June 20	10,230,000
94	Aug. 26	464	34.8	79.0	52.9	trace	161.9	15.0	Aug. 28	14,860,000
Unclassified soil										
105	Sept. 3	488	41.3	29.3	18.7	trace	33.4	8.7	Sept. 8	4,662,000

well as with water-rounded boulders and pebbles. The size and vigor attained by sugar maple, elm and apple trees on these soils is noteworthy. Orchard soils are also characterized by the depth to which organic matter persists in their subsoils and by their extremely high content of organic matter. Calcium and magnesium are not usually lacking to any considerable extent and there is a naturally large supply of phosphorus and potassium. In both surface soils and subsoils the ratios between silica and the sesquioxides, and between carbon and nitrogen, are relatively low, which indicates the desirable condition existing in the soil colloidal complexes.

It is of interest to speculate as to the origin of the organic matter which persists to such depths in these soils. It is very well decomposed and is mixed with rock fragments. Since these soils are on slopes, the possibility suggests itself that the organic materials may be tree debris that has descended from further up on the slope. Another hypothesis is that they may be in considerable part the remains of plants and of surface soils high in organic matter, pushed into this position by glacial or water action. In some cases these soils contain essexite and syenite rock fragments, in others limestone fragments and in still others red shale or sandstone fragments rich in potash. In all instances the high total mineral content balances and helps to modify the organic matter favorably. In view of their already high content and excellent balance of total fertility elements, both acidic and basic, it is predictable that new and profitable apple orchard practices will be developed on these soils.

TABLE XXII

PERCENTAGES OF SOME CONSTITUENTS OF UPLAND PODSOL SURFACE SOILS AND SUBSOILS

Sample number	Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂	CaO	MgO	K ₂ O	C (organic)	N	P ₂ O ₅	SO ₂	Ratio C/N	Ratio SiO ₂
										Fe ₂ O ₃ + Al ₂ O ₃ + TiO ₂
Appalachian region										
78	19.37	0.53	1.05	1.42	4.94	0.33	0.16	0.16	14.8	3.33
78A	16.83	0.69	1.34	2.08	0.87	0.07	0.06	0.06	12.1	4.32
79	16.72	0.59	0.65	1.89	6.62	0.30	0.16	0.13	22.3	4.04
79A	17.98	0.87	1.46	1.74	2.09	0.15	0.13	0.08	14.0	3.79
107	11.87	0.29	0.54	1.34	5.17	0.24	0.09	0.13	21.5	6.08
107A	17.39	0.81	1.20	1.11	0.67	0.03	0.17	9.07	25.8	4.34
109	10.09	0.37	0.43	0.83	5.22	0.21	0.08	0.13	25.2	7.17
109A	17.18	0.63	0.96	0.82	1.75	0.06	0.06	0.09	30.7	4.26
110	17.41	0.65	0.78	1.75	3.26	0.22	0.18	0.17	15.0	3.96
110A	18.97	1.25	0.94	1.65	0.80	0.04	0.16	0.08	21.1	3.84
111	17.67	0.61	0.64	1.49	3.70	0.21	0.10	0.12	18.0	3.88
111A	19.89	0.72	0.77	1.33	1.63	0.09	0.15	0.11	18.5	3.57
Laurentian region										
97	20.91	1.89	1.17	2.17	6.28	0.25	0.19	0.14	29.3	2.86
97A	22.26	2.33	1.90	2.52	2.23	0.10	0.26	0.10	25.6	2.77
38	18.01	2.62	1.52	1.99	7.90	0.31	0.19	0.14	25.7	3.55
38A	18.95	3.27	2.01	2.18	0.83	0.15	0.21	0.09	5.5	3.51

Brown Earth Soils

The brown earths, as they occur in the upland Eastern Townships region of Quebec, have been briefly described elsewhere (8). They occur very widely distributed throughout the region under discussion and may be defined as intermediate in several respects between the orchard soils and the upland podsols. Their fertility is far less than that of the orchard soils though they are quite well supplied with organic carbon and nitrogen. The leached, ash-like layer characteristic of the podsols is absent, the constituents leached from the upper layers being uniformly distributed throughout the upper foot or so of the soil.

They contain in many cases large and small fragments of rock. These fragments, which may be of slate, shale, sandstone, limestone, etc., are crumbling, and in many cases supply the soil with useful minerals. The better mineral supply undoubtedly helps to differentiate these soils from the upland podsols and to obviate the worst effects of leaching.

The brown earths usually occur at lower elevations above mean sea level than the upland podsol soils and are ordinarily more fertile. It will be observed that the brown earths are distinctly better supplied with the oxides of iron, aluminium and titanium, and with phosphorus, potassium, calcium

TABLE XXIII

PHYSICAL AND BIOLOGICAL CHARACTERISTICS OF UPLAND PODSOL SURFACE SOILS

Sample number	Date collected	Period incubation, days	Water-holding cap'y, fresh soil, %	Moisture in fresh soil as % of water-holding cap'y	Moisture in soil after incubation as % of water-holding cap'y	Nitrate nitrogen in fresh soil, parts per million	Nitrate nitrogen after incubation, parts per million	Carbon dioxide, mgm. per 100 gm. soil per 100 hr.	Date plated	Bacteria and actinomyces per gm. soil
Appalachian										
78	June 25	449	63.9	78.7	81.4	0.00	188.2	25.1	July 2	8,256,000
79	June 25	449	70.9	74.2	63.3	trace	159.2	19.9	June 28	4,063,000
107	Sept. 26	465	50.8	104.2	83.6	0.00	92.4	11.1	Oct. 6	5,140,000
109	Sept. 26	465	43.7	100.5	84.1	0.00	59.5	15.1	Oct. 6	3,495,000
110	Sept. 27	465	43.3	93.6	69.8	0.00	154.4	21.5	Oct. 6	4,312,000
111	Sept. 27	465	49.9	73.1	42.4	0.00	139.3	10.6	Oct. 6	4,415,000
Laurentian										
97	Aug. 26	464	56.0	73.4	61.5	trace	154.1	8.7	Aug. 28	10,510,000

and magnesium than are the upland podsols. Their contents of acidic and basic constituents are also better proportioned.

The ratios of carbon to nitrogen, magnesia to lime and of silica to the combined oxides of iron, aluminium and titanium, are all considerably narrower than are the corresponding ratios in the upland podsol soils.

Upland Podsols

This group of soils has been previously described at some length (8), but because of its wide distribution it will be discussed further. These soils are the highest, except the orchard soils, in their content of organic carbon. They are quite well supplied with total nitrogen, total phosphorus and total sulphur. The basic mineral constituents, however, are lacking to a very considerable extent. Not only are the "cementing" bases, iron, aluminium and titanium, deficient, but it will be seen that calcium, magnesium and potassium are very deficient in both surface soil and subsoil. It will be recalled that the lowland podsol soils as a group show quite different deficiencies in total quantities of elements, than the upland podsols. Also, the subsoils of the lowland podsols compare favorably with the subsoils of the heavy clay and sandy clay soil groups in mineral fertility elements.

The upland podsol subsoils are better supplied with mineral fertility elements than are the surface soils. This fact is reflected in the ratios between silicon dioxide and the combined iron, aluminium and titanium oxides of the surface soils and subsoils. The suggestion has been made previously, (8), that the better mixing of surface soils and subsoils through the practice of deep ploughing should, in the course of time, effect an improvement in the fertility of these soils.

The outstanding characteristic of the upland podsol group is the high content of organic matter compared to that of the significant base elements. The club moss hummocks existing on these soils in their virgin state, and the cold, wet nature of their surface soils, which show poor drainage in the presence of small quantities of mineral colloids, reflect the state of this organic matter. The agricultural soils in the area of which the upland podsols are characteristic are in most cases, light brown or almost yellow-brown in color, instead of being dark colored, as they should be, considering their high content of organic matter. It is in this high content of organic matter that hope lies for the improvement of these at present infertile upland podsol soils. If the weight of an acre of surface soil to a depth of 8 in. be estimated at 2,000,000 lb. (and this is probably a conservative estimate), then, with an average nitrogen content of 0.27%, these soils contain on the average 5400 lb. of total nitrogen per acre to a depth of 8 in. This is a tremendous store of nitrogen and, if feasible and economic methods for its release can be evolved, it is likely that the fertility of the upland podsols may be greatly increased, even in the absence of high total contents of calcium, magnesium and potassium.

The ratio of MgO/CaO is very much higher in these upland podsol soils than in any of the other groups, and it would seem that the influence of magnesium must be a highly significant factor in these soils.

The upland podsols of the Appalachian region are quite unlike those of the Laurentian highlands. In the case of the latter, the soils of the upland podsol group have not been chemically analyzed to any extent, although they have been studied in the field, but, from the evidence that we possess, they present problems very different from those of the Appalachian region. Their climatic and geological environments have been dissimilar.

Discussion of Microbiological Factors in Surface Soils

Moisture Relations

Microbiological activity is dependent to some extent on the amount of water present in the soil. With regard to the soils under consideration, the moisture content of the samples in the fresh state has been expressed in Tables XIV to XXIII as a percentage of the water-holding capacity. The averages of these values for the different groups of soils studied were as follows: upland podsol, 85.4%; lowland podsol, 85.3%; sandy clay, 74.2%; and heavy clay, 62.2%. From these figures it appears that the heavy clay soils, at the time of sampling, contained about the optimum percentage of moisture for some phases of microbiological activity. The average water-holding capacity for each group, expressed as percentages of oven dry soil, was as follows: heavy clay, 55.0%; upland podsol, 54.1%; sandy clay, 46.4%; and lowland podsol, 39.7%. The high value of the upland podsol group must be due to the amount of organic matter present.

Organisms

A first consideration with regard to microbiological activities in soil is the well-established fact that these are affected by season as well as by soil con-

ditions. The samples used in this investigation were collected between June 10 and October 6, 1930. It is not known to what extent seasonal factors influence these activities in virgin soils under the climatic conditions of this area. Exact comparisons between fresh soils in any one group are not therefore possible; this is particularly true with regard to numbers of micro-organisms. Recent work (2, 9, 12) has shown that, in cultivated soil, seasonal changes or even fluctuations at intervals of a day or less, amounting in some cases to as much as 100%, may commonly occur.

The numbers of micro-organisms (bacteria and actinomycetes) found in these samples during the season suggest that soil type rather than season is the dominant factor. Averages should, therefore, be suggestive of actual differences, if these exist, between the different soil groups. The highest average and the highest maximum numbers are found in the heavy clay soils; the lowest average and the lowest maximum numbers in the lowland podsol soils. The average numbers found in the latter soils are only one-quarter of those found in the heavy clays. The upland podsol soils contain about twice the numbers present in the lowland podsol soils. The average numbers found in the sandy clay soils are intermediate between those of the lowland podsol soils and the heavy clay soils.

Numbers of Fungi

Counts of fungi were made on 34 of the surface soil and 34 of the subsoil samples. No relation is apparent between numbers of fungi present in the samples and the arrangement of soils in the groups, nor is any apparent between numbers of fungi and season, or between numbers and the locality from which the soils were sampled. There are, however, nearly always more fungi present in the surface soil samples than in the subsoils; in several instances no fungi at all were found in the subsoils.

Nitrate Nitrogen

There was little or no nitrate nitrogen in any of the soils in the fresh state.

Nitrification occurred in all of the samples after incubation, with the original moisture content, at room temperature. The moist samples were stored in glass containers that permitted aeration, and nitrates were determined once, at times varying between 399 and 488 days from the time of sampling. Nitrification was extensive though variable in all the groups of soils studied. On the average, nitrification was highest in the heavy clay soils and lowest in the lowland podsol soils. Variation between soils of the same group was high, but this does not appear to be due to length of incubation.

In the case of seven samples: namely, Nos. 62 and 89 (sandy clays); 78, 107 and 109 (upland podsols); and 86 and 93 (lowland podsols), the percentage saturation of the fresh soil remained above 79.5% while nitrification was proceeding. Greaves (5) has found that with moisture at 80% of the water-holding capacity of the soil, nitrification will proceed only as far as one-tenth of the maximum nitrification that takes place at the optimum moisture content, namely 60% of saturation. The moisture contents of the rest of the

samples remained between 40 and 79.5% of saturation during the course of nitrification. A test of nitrifying capacity was made after the soils had been air-dried and remoistened to 60% of saturation in all samples. Determinations of nitrate nitrogen, made at varying periods up to 135 days, showed that nitrification was greater than assimilation of nitrate in the case of the heavy clay soils, and the sandy clay soils, but remained stationary in the two podsol groups. In only one soil, No. 83, an orchard soil, was any considerable re-assimilation of nitrate nitrogen observed. In this soil, during the time occupied in drying, nitrates increased from 496 to 649 p.p.m.; 77 days after remoistening the nitrate content had fallen to 412 p.p.m.

Carbon Dioxide

Tables XIV to XXIII show the amounts of carbon dioxide evolved after the air-dried samples had been remoistened to 60% of their water-holding capacity. The differences between the soil groups are not great. The heavy clays, on the average, are the most active, while the lowland podsoles produce the least amount of carbon dioxide. The mean value for the sandy clay soils, 23.5 mgm., is largely due to two samples only, Nos. 62 and 82, which evolved 66.8 and 46.3 mgm. respectively; the average of the remaining 11 samples in this group is 17.6 mgm., a figure nearer to the mean of the two groups of podsol soils.

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SPECIFIC HEAT AND DISSOCIATION OF SIMPLE HYDROCARBONS¹

BY R. RUEDY²

Abstract

The specific heat at constant volume of methane, ethylene and acetylene has been computed from the vibrational frequencies involved in Raman effect and infra-red bands, and is found to agree with experiments of recent date. From the overtones of the infra-red methane band of 3022 cm^{-1} a heat of dissociation into probably CH_2 and H of 101 cal. per mole may be deduced. The number of collisions in which the energy could be transferred from molecule to molecule is too small, however, even at $1,000^\circ\text{C}$., to account for the splitting up of methane into CH_2 and H in quartz tubes where in the initial stage catalytic effects are small. It is likely that the slower and the symmetrical vibrations lead to a lower level of dissociation into CH_2 and H_2 , and that the finite time during which the molecule remains in these vibrational states influences the results. The need for studying the velocity of ultrasound in gases in order to gain a better knowledge of thermal decomposition processes is pointed out.

The Specific Heat of Methane

In several fields of practical importance a reliable knowledge of the specific heat of methane and other hydrocarbons is required. Despite the fact that large masses of methane are recovered or treated at high temperatures, attention had repeatedly to be called to the fact that there existed a serious disagreement between the theoretical and experimental values of the specific heat of the gas (11). It will be shown that the difference becomes smaller when more probable frequencies of vibration of the methane molecule are taken in place of those hitherto used.

The energy which has to be furnished to the rotational motion of the molecule when the temperature of the gas is increased reaches its full value, $\frac{3}{2}R$ per mole (where $R=1.985\text{ cal.}$), at 80°K ., so that, as methane boils at 111.5°K . (-161.6°C .), the specific molecular heat at constant volume of the vapor or gas will be at least $3R$ cal. when measured above 100°K . At this low temperature practically no energy is used up for increasing the vibrations of the atoms (1, 12).

The methane molecule may be represented as a tetrahedron having the carbon in its centre and the hydrogen atoms at the corners, each C-H linkage being formed of a pair of electrons having opposite spins, one contributed by the carbon atom and one by a hydrogen atom (3, 6, 16 p. 98, 14, 18, 22). This model yields nine degrees of freedom for the vibrational motions, and these motions may be resolved in the following way. First, there is f_1 , a vibration in which all the hydrogen atoms oscillate along the lines joining them to the carbon atom. The shape of the molecule remains unchanged, though of course the lengths of the sides change. The centre of gravity of

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atoms and charges remains fixed during the displacement and lies within the carbon atom, the motion being perfectly symmetrical. Second, there is $f_{2,3}$, a less symmetrical vibration of the hydrogen atoms upon the surface of the sphere passing through the corners of the model, with the carbon atom stationary in the centre. Such a motion on a surface involves two degrees of freedom possessing, however, the same energy, or the same frequency. Third, there are the triple frequencies $f_{4,5,6}$ and $f_{7,8,9}$ in which the carbon atom changes its position with respect to the hydrogen atoms. Such a motion in three dimensions alters the position of the centre of the electrical charges, and leads to the emission and absorption of radiant energy. The strong infra-red bands at wave-numbers 1304 (7.7μ) and 3022 (3.3μ) correspond to these displacements.

On the other hand, Raman scattering in methane gives three lines or bands which may be used for determining vibrational frequencies: one very strong line at 2914.8 (intensity 20), a very weak one (intensity 2) at 3071.5 and a band of lines between 3261.2 and 2814 cm^{-1} , which latter corresponds to the well-known infra-red emission at 3.3μ (4). The strong lines with the wave-number difference 2915 are due to the perfectly symmetrical oscillation f_1 of the hydrogen shell. The $f_{2,3}$ vibration predicted to give Raman lines at about 1520 cm^{-1} from the exciting radiation (7, 13) fails to appear on the plates; but it is known that Raman scattering is unlikely to produce with great strength vibrational transitions involving slight changes in the electrical moment of the molecule (16). On the other hand, the frequency 1520 cm^{-1} seems high in view of the fact that in CBr_4 and CCl_4 the ratio $f_{7,8,9}/f_{2,3}$ is 1.49 and 1.46 respectively, so that for methane a value between 900 and 1304 is much more probable for $f_{2,3}$ than 1520 cm^{-1} . The shift of 1100 cm^{-1} seems to explain the infra-red bands observed (Table I) to the same extent, but, as the older value is still more or less generally accepted, the computation will be given for both frequencies. There seems, on the whole, sufficient reason for establishing the following correspondence between the possible frequencies of vibrational motions: f_1 , 2915; $f_{2,3}$, 1100 (1520); $f_{4,5,6}$, 3022; $f_{7,8,9}$, 1304 cm^{-1} .

TABLE I
INFRA-RED BANDS OF METHANE (7)

Combination	λ in μ	Combination	λ in μ
$2f_1^*$	3.54—3.84	$2f_7+f_4$	1.77
$2f_4$	1.69	f_4+f_1	3.84
f_7+f_1	2.37	f_4+2f_1	1.15
f_4+f_2	2.42	$2f_4+f_2$	1.4
f_4+f_7	2.32	f_7+2f_4	1.36
$2f_7+f_1$	1.80	$2f_4+f_1$	1.11
		$3f_4$	1.15

* In this table f_4 stands for $f_{4,5,6}$, and f_7 for $f_{7,8,9}$.

The part of the specific heat per mole which must be allotted to vibration of the atoms is given by the formula

$$C_v = R \sum_i n_i \left(\frac{hf_i}{kT} \right) \frac{\frac{hf_i}{kT}}{\left(\frac{hf_i}{kT} - 1 \right)^2},$$

where n_i is the number of degrees of freedom of the corresponding vibrational frequency f_i ; T is the absolute temperature, and $h/k = 4.8 \times 10^{-11}$ deg. sec. When, as usual, the frequencies are indicated in wave-numbers, multiplication by $\frac{hc}{k} = 1.432$ gives the value of $hf/k = \theta$. At very high temperatures it may become necessary to consider harmonic, as well as fundamental, frequencies, at least when dealing with slow vibrations. The fraction of methane molecules

TABLE II
VIBRATIONAL ENERGY OF METHANE IN CALORIES

Temp., °K.	173	273	373	473	573	673	773	f, cm^{-1}
Vibration	Vibrational energy, cal.							
f_1				0.023	0.071	0.155	0.264	2,915
$f_{2,3}$	0.0185 (0.0010)	0.206 0.040	0.535 0.190	0.847 0.414	1.098 0.654	1.282 0.868	1.42 1.051	1,100 1,520)
$f_{4,5,6}$			0.003	0.023	0.071	0.155	0.264	3,022
$f_{7,8,9}$	0.0040	0.098	0.339	0.621	0.876	1.107	1.237	1,304

TABLE III
MOLECULAR HEAT, C_v , OF METHANE

Molecular heat, cal.			
Temp., °K.	Computed from $f_{2,3} = 1100 \text{ cm}^{-1}$	Computed from $f_{2,3} = 1520 \text{ cm}^{-1}$	Experimental
173.0	6.05	6.01	6.61
273.0	6.71	6.38	
297.7	6.98	6.55	
373.0	8.20	7.40	
473.0	9.65	8.78	
481.2	9.76	8.72	9.23
573.0	11.11	10.22	
673.0	12.51	11.68	

which possess one quantum of energy of vibrational frequency $\nu_{7,8,9} = 1304$ (or 0.16 volt) is 0.0009 at 273° K., 0.03 at 546° K. and 0.17 at 1092° K. Below 800° C. the proportion of these molecules is therefore quite small. (Moreover, at higher temperatures, partial dissociation of methane sets in with absorption of an additional amount of energy which increases the specific heat of the gas. In silica bulbs the first sign of decomposition is found at 690° C. It is therefore not useful to compute C_v much beyond this point.) The contribution which has to be made to each single frequency in the range of temperatures where no appreciable decomposition takes place is listed in Table II. This gives the values in Table III for the molar heat, C_v , of methane (21).

The agreement between theory and experiment (Fig. 1) is therefore as good as in the case of nitrogen and oxygen (8). Fig. 1 contains for comparison the values obtained for water vapor (21), the frequencies used for drawing the curve being 3654, 1648 and 984.

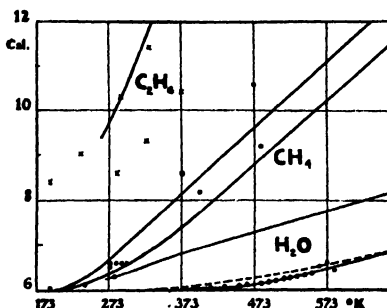


FIG. 1. Molecular heat of methane (upper curve computed with $\nu_2 = 1100$, lower curve with $\nu_2 = 1520 \text{ cm}^{-1}$, experimental values marked by dots). Ethane (experimental values marked by crosses) and water vapor (upper curve from Raman frequencies, dashed line from empirical formula, lowest curve, experimental values).

Vibrational Frequencies and Dissociation

The absorption spectrum of methane gas shows a band in the far red composed of lines lying between 6500Å and 9500Å, which may be identified as the third overtone ($\nu' = 4$) of the fundamental absorption band at 3.3μ . The first and second harmonics lie at 1.69 and 1.15μ , the progression of bands being represented by the formula (2),

$$\nu = 3085n - 66n^2 \quad (\text{cm}^{-1}).$$

A long and therefore somewhat uncertain extrapolation leads to $n = 23$ as the value at which the vibrational frequency falls to zero, so that the molecule falls apart. At this stage the total vibrational energy accumulated would have increased to $35,926 \text{ cm}^{-1}$ or about 101 cal. This is also nearly the energy indicated from thermochemical considerations as being necessary for the removal of one hydrogen atom from the methane molecule (110 to 130 cal.).

As in the case of diatomic molecules, there is, however, no absorption of radiation in the region corresponding to this heat of dissociation; even the absorption of the third overtone becoming appreciable only when columns of great length, about 10 m. at atmospheric pressure, are used (2). On the other hand, the number of collisions in which the relative kinetic energy q of both particles exceeds the value of 115 cal. per mole is negligible, even at temperatures of more than 1,000° C., as it is given by the formula (17),

$$\frac{1}{3} \epsilon - \frac{q}{RT} \left[\left(\frac{q}{RT} + 1 \right)^2 + 1 \right] s,$$

where z is the total number of collisions which a single methane molecule makes per second ($z=1.25 \times 10^{10}$ at 273° K., $z=1.75 \times 10^{10}$ at 546° K., $z=2.5 \times 10^{10}$ at 1092° K., and $z=3.5 \times 10^{10}$ at 1373° K.). It may be seen from Table IV, which gives the proportion of collisions in which the relative

TABLE IV
COLLISIONS OF METHANE MOLECULES IN WHICH THE RELATIVE ENERGY
EXCEEDS q CAL. PER MOLE

Temp. °K.	q , calories				z
	115	69	46	23	
273				2.9×10^{-15}	1.3×10^{10}
546		1.3×10^{-28}	2.1×10^{-20}	0.9×10^{-9}	1.8×10^{10}
819	3.2×10^{-26}	2.7×10^{-14}	1.9×10^{-10}	0.6×10^{-4}	2.2×10^{10}
1,092	1.3×10^{-18}	10^{-10}	0.8×10^{-9}	1.1×10^{-3}	2.5×10^{10}
1,365	1.7×10^{-14}	10^{-8}	0.5×10^{-5}	0.6×10^{-2}	3.5×10^{10}

energy exceeds the value q cal. per mole, that even at 1100° C. a methane molecule collides with a second molecule possessing a relative energy of 115 cal. per mole only once in about 1600 sec., and then it is by no means likely that the whole energy can be used up for increasing the vibrational motion. On the assumption that a particle at rest acquires the internal energy q from the impinging methane molecule, the laws governing the impact between neutral particles demand that it takes up at the same time an equal amount of translational energy, the velocity v_2 of the second particle after the collision being given by the following formula:

$$v_2 = \frac{u_1 + \sqrt{u_1^2 - 4q/M}}{2}$$

where u_1 is the velocity of the first molecule before it hits the second and M the mass of the methane molecule (10, 24). That is, in order to take up the energy q as internal energy, a molecule must be hit by a particle of the same mass, but possessing at least the kinetic energy $2q$. In practice, the experiments on thermal dissociation are carried out in fairly small vessels or narrow tubes (5, 23), sometimes of metal, but more often of quartz or porcelain, in which case catalytic effects may be rendered small, at least during the initial stage, but where the methane molecules may pick up energy from the vibrating atoms forming the solid wall. At the high temperatures employed, the exact frequency distribution of these oscillators may be deduced from Born and Karman's theory of specific heat. Taking into consideration only the average energy of the molecules forming the wall of the vessel would change the number of collisions from z to z_w , the number of molecules striking normally 1 sq. cm. per sec. being $z_w = \frac{nC}{\sqrt{\pi}}$, where n is the number of molecules per

cubic centimetre, and C , the mean square velocity of the molecules, is equal to $60,060\sqrt{T/273}$ cm. per sec. These collisions become important only at low pressures and in narrow tubes (17, 23).

It seems quite possible, however, that the overtones of the symmetrical mode of vibration 2914 cm^{-1} converge to a lower limit of dissociation than those of the infra-red band. The vibrational levels of nitrogen as compared with those of carbon monoxide may be cited in support of this assumption. The energy required for removing a second hydrogen atom from CH_3 has been estimated at 20 to 40 cal. per mole, so that if the molecule can be decomposed in a single step into CH_2 and H_2 , only about 50 to 60 cal. per mole would be required. This, at any rate, is one value which has been observed for the heat of activation in the thermal decomposition near a solid wall (17). Unfortunately no overtones have been established for the symmetrical vibrations, and strong Raman lines are theoretically restricted to the fundamental frequency differences, at least in the lighter molecules. Overtones could be obtained only by means of very long exposures and under the influence of strong external forces. The vibrations $f_{2,3}$ and $f_{7,8,9}$, on their side, definitely point to a smaller force holding the atoms in place, and therefore to the existence of a lower limit of breakdown, so that as far as the vibrational motions of the atoms are concerned, there is little likelihood that CH_3 and H form the first products in the decomposition of the methane molecule by heat (9).

Work on the velocity of ultrasonic waves in gases has in addition called attention to another factor which must be taken into account when methane is rapidly drawn through hot quartz tubes, namely, the finite time during which a molecule remains in its vibrational state (9). Towards frequencies of over 10^6 cycles per sec. a compound gas behaves as if it had one or several degrees of vibration less than in the normal state, because there is not sufficient time to excite the slow vibrations which it is capable of performing before the adiabatic compression of the cycle has already vanished. This results in a persistence of the vibrational state of the order of 10^{-6} sec. in the case of the infra-red carbon dioxide band $14.7\text{ }\mu$ (675 cm^{-1}), and probably more for the symmetrical motions.

During this time a molecule suffers a large number of collisions and may at times accumulate excess energy before the steady state has become established, a fact not usually considered in the kinetic theory of chemical reactions. It will be of great interest therefore to extend the study of the velocity of ultrasonic waves to gases in which the slowest vibrations are perfectly symmetrical, and are sufficiently well excited at ordinary temperatures, as in the case of chlorine. Chlorine molecules are capable of only one type of vibration, a symmetrical vibration of frequency 560 cm^{-1} , and at 0° C. , a fraction of $e^{-802/T}$, or about 5.3% of the total number of the molecules are in this state (9). In other words, one molecule possesses the corresponding energy of vibration during about 0.05 sec. as against 0.03 sec. in the case of the lowest, active vibration (675 cm^{-1}) of CO_2 . A still larger influence on the velocity of high-frequency sounds may be expected in the cases of ethane, ethylene, acetylene and other hydrocarbons as a discussion of their specific heats will show.

Specific Heat of Ethane, Ethylene, and Acetylene

In contrast to methane, the molecules of ethane, ethylene and acetylene possess several frequencies of vibration which are fairly well developed at room temperature, and which are likely therefore to influence the velocity of high-frequency sound waves. In the first group of oscillations the carbon and its attached hydrogen atoms in one-half of the molecule move with respect to the atoms in the second half of the molecule, the possible displacements involving seven degrees of freedom represented by five distinct frequencies (3). There is first the symmetrical vibration of the carbon atoms, with the hydrogen atoms remaining practically in place (frequency ν_1); second, that of one hydrogen

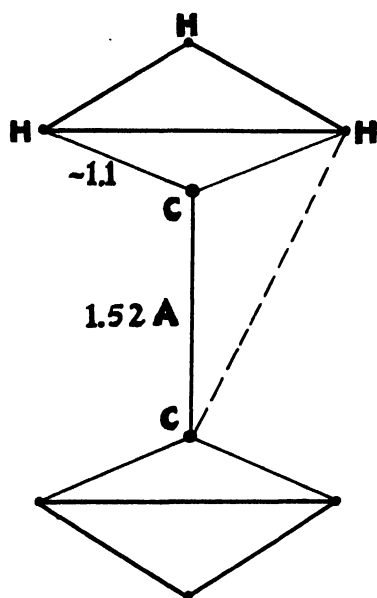


FIG. 2. Structure of the ethane molecule.

group toward the other hydrogen shell along the line of least inertia, that is, the line joining the carbons, (inactive frequency ν_2); third, the optically active vibrations of the C_2 radical as a whole towards the two H groups moving in phase, the change of the electric moment taking place either along the axis joining the two halves of the molecule (frequency ν_3), or in a plane perpendicular to it (active frequency ν_4); and finally, the nearly symmetrical vibrations of the hydrogen group with respect to the carbon to which it is not directly bound, the displacement taking place normally to the axis (double frequency $\nu_{5,6}$). In the case of the acetylene molecule where the atoms are strung out along the same line, ν_4 is a double frequency, because the C_2 group may move not only along a line, but in a plane perpendicular to the figure axis (2).

This exhausts all the possibilities in the case of acetylene, where indeed five different frequencies have been established (13): ν_1 , 1980; ν_2 , 3320; ν_3 , 3277 (3μ); $\nu_{4,5}$, 730 (13.7μ); $\nu_{6,7}$, 590 cm^{-1} . In view of the great stability of the molecule, it must be assumed that the overtones are nearly harmonic. In the case of ethane and ethylene, additional optically active oscillations are possible within each half of the molecule. Each $-CH_3$ pyramid, for instance, possesses four distinct frequencies representing six degrees of internal freedom (Fig. 2). These are first the vibrations of the hydrogen atoms alone, approximately along the lines pointing to the centre of gravity of the H_3 triangle, and causing a slight displacement of the carbon atom, such that the electric moment oscillates along the axis of symmetry (frequency ν_7) or in the plane perpendicular to it (double frequency $\nu_{8,9}$). A last group of vibrations corresponds to the motion of the three hydrogen atoms moving now as a whole with respect to the carbon atom to which they are bound, the plane of the triangle remaining parallel

to itself. The carbon atom may move along the axis (frequency ν_{10}) or in the plane perpendicular to it (frequency $\nu_{11,12}$). As all the frequencies appearing in one pyramid also belong to the other half of the molecule, the total number of frequencies for ethane is 18. Resonance effects between the two halves may influence somewhat the frequencies of each half of the molecule. In the case of ethylene, where all the atoms lie in the same plane, each triangle possesses three kinds of oscillations, the total number of frequencies being 12, in agreement with the formula giving the total number of degrees of freedom of a molecule composed of n atoms as equal to $3n - 6$.

The frequencies of vibration are then, for C_2H_2 as shown above; for C_2H_4 , $\nu_1 = 1623$, $\nu_2 = 3019$, $\nu_3 = 2880$ (3.3μ), $\nu_4 = 950$ (10.5μ), $\nu_{5,6} = 700-780$, $\nu_7 = 1342$, $\nu_8 = 3272$, 3240 , $\nu_9 = 1444$ (6.9μ); for ethane, C_2H_6 , $\nu_1 = 990$, $\nu_2 = 2955$, $\nu_3 = 2950$ (3.4μ), $\nu_4 = 825$ (12.2μ), $\nu_{5,6} = 825$, $\nu_7 = 2900$, $\nu_{8,9} = 975$, $\nu_{10} = 2950$ (3.4μ), $\nu_{11,12} = 1450$ (6.8μ). The number of strong Raman frequencies observed is smaller than expected, a feature common to heavier molecules where all the oscillations tend to become active (1). Still lower Raman frequencies would appear if molecules with long carbon chains were examined, until at the limit, the vibrations are fully excited at room temperature, the influence of the finite life of the molecule in its vibrational state being then outweighed by that of the large number of collisions capable of causing the molecule to oscillate. The agreement between the computed and the observed values of the specific heat shown in Table V is satisfactory, but there is considerable uncertainty in the values of the frequencies ν_6 which seem to have been taken from combination bands (13). In the case of ethylene the values obtained suggest that the carbon and the two hydrogen atoms lie nearly in a straight line.

TABLE V
SPECIFIC HEATS OF C_2H_2 , C_2H_4 AND C_2H_6

Specific heat, cal. per gm. mol.	Temperature, °C.		
	17	64.4	100
<i>Acetylene</i>			
Obs.	8.4		
Calc.	8.6		9.9
<i>Ethylene (15)</i>			
Obs.	8.1		9.7 to 12.0
Calc.	8.6		
<i>Ethane</i>			
Obs.	10.37	11.4	
Calc.	10.50	11.8	

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THE POLYMERIZATION OF ACETALDEHYDE. II¹.

BY W. H. HATCHER² AND MURIEL G. KAY³

Abstract

Using acetaldehyde prepared out of contact with oxygen, the specific gravities of this substance and its mixtures with paraldehyde have been determined, and shown to differ from those previously obtained. The following values for specific gravity were obtained:— pure acetaldehyde, 0.7865 (15° C.); pure paraldehyde, 0.9984 (15° C.); metaldehyde, 1.120 and 1.127.

The rates of polymerization of acetaldehyde with and without contact with oxygen have been shown to differ, and the latter values are in agreement with a former study.

Historical

In a recent article by Hatcher and Brodie (3), a study was made of the mechanism of the polymerization of acetaldehyde to paraldehyde, phosphoric acid being used as a catalyst. Their findings indicated the reaction to be trimolecular at low concentrations of catalyst, and the velocity to be directly proportional to the quantity of catalyst present. At the same time they observed the necessity of using only freshly distilled material and that *as soon as possible* after distillation. Samples of acetaldehyde gave slow velocities of polymerization when allowed to stand longer than two hours, although no trace of any polymer could be detected in them.

A more recent publication (4) indicated the rapidity with which gaseous acetaldehyde absorbed a small quantity of oxygen from the atmosphere to give rise to a peroxide. Conant (1, 2) has observed an increase in the velocity of polymerization of butyraldehyde due to traces of the peroxide.

In the light of these results it was decided to prepare acetaldehyde in an oxygen-free atmosphere and repeat some of the experiments of Hatcher and Brodie in order to gauge the effect of catalysts excluding oxygen either free or combined.

A careful examination of the specific gravities of mixtures of acetaldehyde and paraldehyde as found by Pascal and Dupuy (6) required redetermination of these values. This has been done, using oxygen-free acetaldehyde.

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Experimental

Preparation of Paraldehyde and Acetaldehyde

Paraldehyde was distilled in the usual way from a commercial sample until a fraction was obtained which boiled at 124° C.

Acetaldehyde was prepared in two stages from commercial paraldehyde and a catalyst—concentrated sulphuric acid. In the first stage fairly pure acetaldehyde was distilled from a flask, through a spiral condenser, and into a collecting flask, the condenser and second flask being surrounded by ice and salt. The second stage was the same except that the final receiver was specially constructed with a tube attached to the bottom so that the acetaldehyde could later be forced out by the pressure of nitrogen in the apparatus. Both distillations were carried out in the absence of oxygen; that is, the apparatus in each case was filled with nitrogen at the beginning, and a small stream of nitrogen was passed through continuously during the process. A carbon dioxide atmosphere was used in two cases, but found to give the same results as nitrogen.

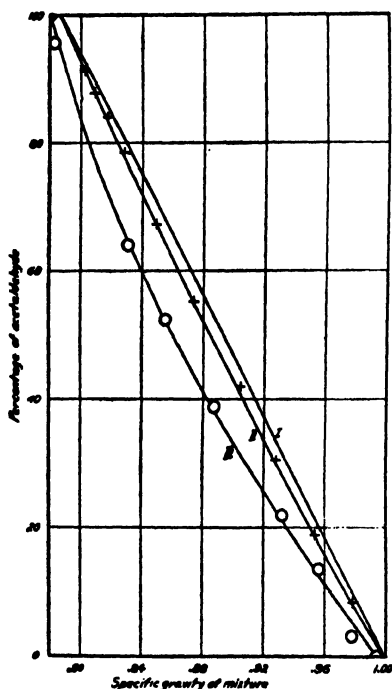


FIG. 1. *Specific gravity curves of acetaldehyde-paraldehyde mixtures.*

varying amounts of paraldehyde were weighed into the pycnometer and enough acetaldehyde added so that the total volume would be a previously calculated one. The weight of acetaldehyde put in the bulb was then found.

Thus the percentage composition of the mixtures and their specific gravities were determined.

In the first stage, the temperature of the water bath around the starting flask was kept at or below 50° C. in order to hasten the distillation, but in the second the temperature was kept at about 30° C.

Cleanliness of all apparatus concerned with the preparation of materials and with subsequent experimentation was strictly observed. Each piece was washed with alcohol, then thoroughly with water, and dried before each experiment.

Determination of Specific Gravities of Mixtures of Acetaldehyde and Paraldehyde

The apparatus used consisted of a pycnometer, constructed in the form of a bulb of about 80-cc. capacity attached to a narrow tube with a stopcock and side arm at the top. A thermostat was used and kept at 15° C.

The specific gravities of pure acetaldehyde and pure paraldehyde were measured by weighing such an amount of material that the level was in the narrow tube. The bulb was then calibrated with water at this level. For mixtures of acetaldehyde and paraldehyde,

These results are given in Table I and shown graphically in Curve II of Fig. 1.

TABLE I
SPECIFIC GRAVITIES OF ACETALDEHYDE-PARALDEHYDE MIXTURES

Wt. of acetaldehyde, gm.	Wt. of paraldehyde, gm.	% Acetaldehyde	Total volume, cc.	Specific gravity
66.7574	0.0	100.00	84.879	0.7865
62.1869	5.9883	91.22	84.879	0.8032
60.3126	8.3903	87.78	84.879	0.8095
58.2204	11.1673	83.90	84.879	0.8175
55.2129	15.1132	78.51	84.879	0.8285
48.5633	23.5317	67.36	84.879	0.8494
40.9614	33.2090	55.23	84.879	0.8738
32.2622	44.4950	42.03	84.879	0.9042
24.1411	54.6611	30.63	84.879	0.9283
15.2879	65.6268	18.89	84.879	0.9532
6.9656	76.0483	8.40	84.879	0.9781
0.0	84.9150	0.0	85.05	0.9984

I is a straight line drawn between the specific gravities of pure acetaldehyde and pure paraldehyde and represents the ideal curve for the specific gravities of mixtures of the two. III shows the results obtained by Pascal and Dupuy for such mixtures. I and II refer to specific gravities at 15° C., while III refers to specific gravities at 20° C.

Specific Gravity of Metaldehyde

During the preliminary experiments on the polymerization of acetaldehyde, metaldehyde was almost always formed along with paraldehyde. It was therefore decided that since the specific gravity of metaldehyde would be an important factor in studying the rate of polymerization, it should be determined. This was done by weighing about 1 gm. of metaldehyde in a small specific gravity bottle against water for comparison. Since metaldehyde is insoluble in water, the true weight of water displaced was measurable. Two specific gravity determinations were made and the values found to be 1.120 and 1.127.

Polymerization of Acetaldehyde

When these preliminary data had been obtained, acetaldehyde was subjected to polymerization. Above 10° C., paraldehyde is the usual polymer, while below 10° C. quantities of metaldehyde are commonly obtained also. The dilatometer used was the same as that used by Hatcher and Brodie although the scale reading and the total volume were slightly different. One division on the scale (from a Beckmann thermometer) corresponded to 0.0079 cc. and the total measurable capacity of the dilatometer was 26.071 cc.

In each experiment the catalyst was 0.10 cc. of 85% phosphoric acid. In each case the dilatometer was filled with nitrogen before allowing the acetaldehyde to pass into it; it was then filled with acetaldehyde and allowed to stand for a few minutes in the thermostat. The procedure for studying the polymerization was the same as that of Hatcher and Brodie. The rate of polymerization was measured by watching the decrease in volume of the

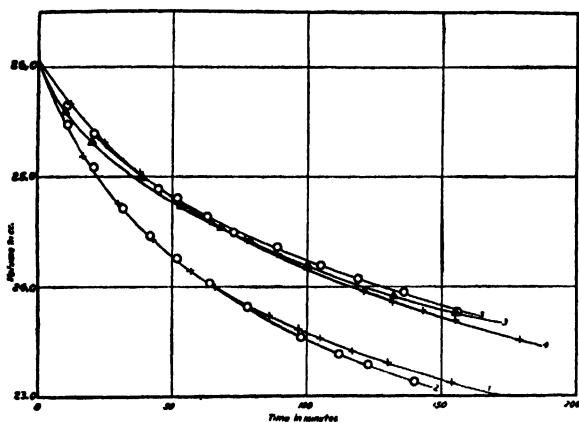


FIG. 2. Volume-time curves (acetaldehyde-phosphoric acid).

duced for a definite length of time.

Curve No. 1 (Fig. 2) is the typical oxygen-free experiment and showed no formation of metaldehyde. No. 2 confirmed No. 1 by coinciding with it up

mixture in the dilatometer, readings of the level of the liquid being taken at intervals of about 10 min.

The results of five typical polymerization experiments are given in Table II and shown graphically in Fig. 2.

From the beginning of the preparation of the acetaldehyde to the end of the polymerization, the work was done with minimum oxygen interference except in cases where oxygen was purposely intro-

TABLE II
RESULTS OF FIVE TYPICAL POLYMERIZATION EXPERIMENTS
(0.10 CC. 85% PHOSPHORIC ACID AS CATALYST)

Expt. No.	1	2	3	4	5	Expt. No.	1	2	3	4	5
Time, min.	Total volumes of mixtures, cc.					Time, min.	Total volumes of mixtures, cc.				
0	26.058	26.058	26.058	26.058	26.058	78		23.817			
10	25.453		25.587			79			.418		
11		25.469			25.650	86	.738			*	
12				25.666		89				.291	.355
17	.176					97	.612				
20			.311			98		.541			
21		.081			.390	100			.181	.157	
25				.303		105	.533				.197
30	24.758					112		.391			
32		24.780				117	.415				
38				.034		119					.070
39			24.987			121				23.960	
42		.473				123		.288	*		
43	.426					130	.304				
45					24.884	132			23.904	.857	
52		.252		24.789	.797	136					23.944
53			.734			140		.130			
57	.149	*				143				.778	
63					.647	154	.122				*
64		.031		.600		155			.754	.691	
66	23.999					156					.770
68			.544			167	.035				
73					.497	179				.517	
77				.426							

*This indicates the moment at which metaldehyde appeared.

to 65 min., then separating from it. This separation coincided with the formation of metaldehyde in No. 2.

Since the oxidation of acetaldehyde after it has been prepared in an oxygen-free atmosphere causes the formation of a peroxide, it was decided to see what effect the passing of oxygen through pure acetaldehyde would have on the rate of polymerization. The results of the typical experiments illustrating this effect are Curves Nos. 3, 4 and 5. In Nos. 3 and 4, oxygen was passed through for one minute, and in No. 5 for five minutes*.

Conant and coworkers, in their work on the pressure polymerization of aliphatic aldehydes, found peroxides to be effective catalysts. It was therefore thought possible that in the above experiments where acetaldehyde peroxide was formed by the addition of oxygen, it acted as a catalyst for polymerization before the phosphoric acid was added; and that the rate at the beginning (the fastest) was not being measured, and this could explain why the experiments with oxygen seemed to progress more slowly than those without. This explanation was subjected to proof by almost filling the dilatometer with pure acetaldehyde and then, when the liquid had reached a constant level, filling the dilatometer to the desired level with oxygenated acetaldehyde. No volume change was observed during a period of over an hour.

TABLE III

POLYMERIZATION OF ACETALDEHYDE WITH 0.10 CC. OF 85% PHOSPHORIC ACID AS CATALYST.
CALCULATION OF BIMOLECULAR AND TRIMOLECULAR CONSTANTS

Time, min.	Volume, cc.	Density, gm./cc.	Acetaldehyde, %	Wt. of acetaldehyde, gm.	Acetaldehyde, gm. mol./litre	Bimolecular const. $K \times 10^4$	Trimolecular const. $K \times 10^4$
Curve No. 1							
0	25.958	0.7865	100.00	20.42	17.87		
10	.353	0.8054	90.18	18.41	16.50	4.645	2.708
20	24.966	0.8177	83.84	17.12	15.58	4.113	2.471
30	.658	0.8279	78.58	16.04	14.79	3.887	2.402
40	.392	0.8371	73.88	15.09	14.06	3.791	2.410
50	.185	0.8441	70.39	14.37	13.51	3.613	2.349
60	.000	0.8507	66.96	13.68	12.94	3.551	2.365
70	23.838	0.8565	64.14	13.10	12.48	3.451	2.347
80	.710	0.8612	61.83	12.63	12.10	3.336	2.312
90	.589	0.8654	59.80	12.21	11.76	3.230	2.277
100	.480	0.8696	57.78	11.80	11.42	3.161	2.270
Curve No. 2							
70	23.833	0.8567	64.02	13.07	12.47	3.464	2.360
80	.685	0.8618	61.56	12.57	12.06	3.371	2.342
90	.546	0.8670	59.00	12.05	11.62	3.344	2.372
100	.425	0.8714	56.94	11.62	11.27	3.275	2.369

*The numerical results of Table II and Fig. 2 include the 0.10 cc. of catalyst which was present in the dilatometer.

From the volume changes, the bimolecular and trimolecular constants were calculated for No. 1 from 10 to 100 min. and for No. 2 after it diverged from No. 1, that is, from 70 to 100 min. These calculations are shown in Table III. Here the volumes referred to do not include the catalyst.

The following method of calculation was used:— as the volume of the mixture at any time was recorded, and as the total weight was constant, the specific gravity of the mixture could be found. From Fig. 1 the percentage by weight of acetaldehyde was obtained, and from this was found the weight of acetaldehyde present, which, with the total volume of the mixture, gave the concentration of acetaldehyde in gram molecules per litre. This value was $(a-x)$ and the concentration of acetaldehyde at the beginning was (a) .

The bimolecular constant was then calculated from the equation

$$K = \frac{1}{t} \times \frac{x}{a(a-x)},$$

and the trimolecular constant from

$$K = \frac{1}{2t} \times \left(\frac{1}{(a-x)^2} - \frac{1}{a^2} \right),$$

where t is the time expressed in minutes.

In order to account for the fact that as the volume of the mixture decreases, the concentration of catalyst increases, the value $(a-x)$ was multiplied by the initial volume and divided by the volume of the mixture at the time represented. This was done for four different values of No. 1, and the trimolecular constants calculated from the new values of $(a-x)$. These constants are shown in Table IV beside the corresponding uncorrected values for purposes of comparison.

TABLE IV
CORRECTED AND UNCORRECTED TRIMOLECULAR CONSTANTS

Time, min.	Without correction	Time, min.	With correction
	Trimolecular constant, $K \times 10^4$		Trimolecular constant, $K \times 10^4$
20	2.471	20	1.708
40	2.410	40	1.670
60	2.365	60	1.638
80	2.312	80	1.603

Discussion of Results

The specific gravity of pure acetaldehyde, as seen from Table I, was found to be 0.7865 at 15° C., and the value at this temperature, as calculated from the value at 18° C. given by Smits and de Leeuw (7) is 0.7870. Since the impurities most likely to be in acetaldehyde are acetaldehyde peroxide and paraldehyde, and since these are both heavier than acetaldehyde itself, a lower specific gravity than that before recorded is acceptable.

The specific gravity of pure paraldehyde, as seen in Table I, was found to be 0.9984 at 15° C., while the value recorded by Kekulé and Zincke (5) is 0.9980.

The agreement in the case of paraldehyde is good, and, although the same

is approximately true in the case of acetaldehyde, the impurities which can be found in the latter substance are such as to increase its specific gravity.

In order to compare easily the values obtained for specific gravities of mixtures of acetaldehyde and paraldehyde with those obtained by Pascal and Dupuy, three graphs were plotted in Fig. 1. No. I is the ideal curve and would be the true one if there were no shrinkage of volume when acetaldehyde and paraldehyde are mixed; II represents the experimental data of Table I; III shows the values obtained by Pascal and Dupuy—done, however, at 20° C. It is seen from Fig. 1 that II is much closer to an ideal straight line drawn between the two ends of the curve than III is. Also the results from which II was plotted are considerably more regular and uniform. This difference may be due to the effect of oxygen.

The specific gravity of metaldehyde is here given for the first time, as a search of the literature reveals no previous value. The values found are 1.120 and 1.127.

The effect of metaldehyde appearing in the dilatometer was, as seen from Curves Nos. 1 and 2 of Fig. 2, to produce a larger contraction in volume and therefore make the rate appear faster than if only paraldehyde were formed. This same effect is observed by comparing No. 3 with No. 4, both of which were experiments in which oxygen was passed through the acetaldehyde for one minute after preparation. The time when metaldehyde was first seen in No. 4 practically coincides with the point where No. 4 crosses No. 3 and continues below it, indicating a faster decrease in volume. This is due to the fact that the specific gravity of metaldehyde is appreciably greater than that of paraldehyde—the first polymer to be formed.

In Table III are seen both the bimolecular and trimolecular constants calculated for No. 1 at 10-min. intervals up to 100 min., and for No. 2 from 70 to 100 min. As in Hatcher and Brodie's work, the trimolecular constants are more consistent and therefore represent more truly the order of the reaction.

Table IV shows a few of the trimolecular constants before and after a correction has been made because of the increasing concentration of catalyst. It is seen that the consistency of the constants is about the same in both cases; that is, the increasing concentration of acid is not an important factor at this concentration since the reaction does not proceed fast enough to make an appreciable difference in the concentration of the acid.

Again, in Fig. 2 is seen the effect of passing oxygen through pure acetaldehyde just before it is used for polymerization. Curves Nos. 3, 4 and 5 are decidedly above No. 1, the standard oxygen-free experiment, and also they are very close to each other. It was shown that the peroxide formed is not alone a catalyst, and in the presence of phosphoric acid it is seen to be a negative catalyst. That is, the general effect of the oxidation of acetaldehyde after it has been prepared free from oxygen is a retardation of the rate of polymerization, and the amount of oxygen passed through seems to be immaterial. This fact is of interest since these data are the reverse of those obtained by Conant and coworkers using butyraldehyde. However, the conditions of their experiments are materially different from those obtaining here.

This work is being extended to ascertain whether these results permit of greater generalization. In addition to the new data provided, these findings indicate the necessity of preparation and use of aldehydes out of contact with oxygen.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLI. STRUCTURE OF RAFFINOSE LEVAN¹

BY WALTER MITCHELL² AND HAROLD HIBBERT³

Abstract

Raffinose levan has been synthesized by the action of *B. subtilis* on raffinose. The product is identical in structure with sucrose levan previously synthesized by the action of *B. subtilis* and *B. mesentericus* respectively on sucrose, and thus represents a polymerized 2:6-anhydro-fructofuranose. The work serves to confirm the theory put forward previously to the effect that levan formation takes place only with sugars containing a terminal fructofuranose grouping. *B. subtilis* and *B. mesentericus* thus exert quite a specific action with respect to the synthesis of levan.

Introduction

In previous communications dealing with the preparation and properties of sucrose levan (1, 2, 3) it has been shown that, irrespective of whether levan is formed from sucrose by *B. subtilis* or *B. mesentericus*, the resulting product in each case is a polymerized 2:6-anhydro-fructofuranose.

In the first communication on this subject (1), the opinion was expressed that, in all probability, with each of these organisms levan formation takes place only with sugars containing a terminal fructofuranose group as, for example, with sucrose, raffinose, turanose, gentianose and stachyose.

In the case of melezitose, which represents a trisaccharide containing a centrally situated, and not a terminal, fructofuranose linkage, no levan formation was found to take place.

In the present communication it is shown that levan prepared by the action of *B. subtilis* on raffinose is identical with that prepared from sucrose, and is therefore a polymerized 2:6 fructofuranose.

Further investigations are under way regarding the structure of "turanose levan" and "gentianose levan".

In view of the remarkable interest attaching to polysaccharides from the point of view of their immunological action this phase of the work is to be investigated by Dr. FitzGerald and coworkers at the University of Toronto.

Experimental

Synthesis of Raffinose Levan

The procedure used in this work is essentially that used by Harrison, Tarr and Hibbert (1). Fifteen per cent raffinose broth was employed, this concentration representing the maximum solubility of the sugar. The yield obtained is much lower than that from sucrose, which can be partly accounted for by the fact that raffinose is a trisaccharide. From 125 gm. of raffinose 11 gm. crude product was obtained, and from the latter about 50% of purified material.

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The method of purification is given below. The yield of pure levan is only about 4.5% or approximately 15% of the fructose fraction. It should be noted, however, that the strain of *B. subtilis* employed continued to show an increasing activity towards raffinose, the last yield obtained being 22%, calculated on the fructose fraction. The unchanged raffinose left in the mother liquor was recovered by evaporation, purified by recrystallization and re-used.

Owing to the rarity of raffinose as compared with sucrose a much smaller amount of material was at the authors' disposal, and some slight modifications in technique had, therefore, to be introduced, in dealing with the raffinose levan. Preliminary experiments with small quantities (3 gm. or less) of sucrose levan showed that it was advisable, first, to methylate the levan using dimethyl sulphate and potassium hydroxide, and then to acetylate this partially methylated product and to follow this by a combined deacetylation and methylation process, rather than to prepare first the triacetate and then deacetylate and methylate this compound. The objections to the latter procedure are that the yield of levan triacetate is frequently poor, and the product is insoluble in acetone rendering interaction more difficult during the subsequent deacetylation and methylation processes. The purification of the crude levan was carried out in exactly the same manner as described in a previous communication dealing with sucrose levan (3). A rotation of a sample of the purified raffinose levan dried at 100° C. over phosphorus pentoxide at about 1 mm. showed $[\alpha]_D^{22} = -45.5^\circ$ in water. An analysis and rotation of the acetate were then made.

Pure white, powdery raffinose levan (0.20 gm.) was added to a mixture of 20 cc. of pure, dry pyridine and 10 cc. of pure acetic anhydride, and the combined products boiled under reflux for about 10 min. A very small amount of a gel remained undissolved and was removed by centrifuging after the mixture had stood overnight at room temperature. The clear solution was poured into about 200 cc. of ice-water and extracted three times with chloroform. After drying over anhydrous magnesium sulphate the solution was concentrated to a gummy residue under 8 mm. pressure, the final temperature reaching 100° C.

The product left was boiled under reflux for two hours with 100 cc. of dry ether. The resulting powdery substance was dissolved in the minimum quantity of methyl acetate and then reprecipitated by dropping the solution slowly into a well-stirred quantity (250 cc.) of dry ether contained in a centrifuge glass. The purified levan triacetate was removed by centrifuging and dried in an Abderhalden vacuum dryer over phosphorus pentoxide at 80° C. Yield, 0.22 gm. $[\alpha]_D^{20} = +8.9^\circ$ in acetylene tetrachloride ($c = 3.01$).

Analysis: Found: C, 49.8; H, 5.9%. Calcd. for $(C_{12}H_{16}O_5)_n$: C, 49.98; H, 5.6%.

Methylation of Raffinose Levan

Purified raffinose levan (5 gm.) was dissolved in 5 cc. of distilled water and stirred vigorously whilst 22 cc. of dimethyl sulphate and 44 cc. of 30% potassium hydroxide were added during 2½ hours. Stirring was continued for a further

two hours before diluting the reaction mixture with about 200 cc. of distilled water. The partially methylated levan was extracted with chloroform five times. This extract was dried over anhydrous magnesium sulphate and then concentrated to a semi-solid at 100° C. under 10 mm. pressure. This was dissolved in 30 cc. of pure dry pyridine, 21 cc. of acetic anhydride added, and the solution boiled gently under reflux for five minutes. After standing for five hours the clear solution was poured into about 500 cc. of ice-cold distilled water and extracted three times with chloroform, the product dried and chloroform removed in the usual manner.

The gummy solid so obtained was dissolved in 20 cc. of pure, dry acetone and methylated with 30 cc. of dimethyl sulphate, and 60 cc. of 30% potassium hydroxide added during two hours, with vigorous stirring, the temperature being raised from 50 to 100° C. during the addition.

The reaction product was then treated in exactly the same manner as in the first methylation. The residual solid was purified by precipitation from its solution in methyl acetate with dry ether. It was then redissolved in benzene and reprecipitated with petroleum ether (b.p. 30–50° C.). The pure white powder was dried under reduced pressure over phosphorus pentoxide at 75° C. and 8 mm. for 12 hr. Methoxyl estimation: 20.20 mg. substance gave 66.5 mg. AgI.—OCH₃, 43.5%.

This partially methylated levan was now first acetylated, and then, in one operation, deacetylated and methylated, using the previously described technique. The methoxyl content remained unchanged.

Recourse to the Purdie method was therefore necessary. The solid was dissolved in 50 gm. of methyl iodide and to this vigorously stirred solution was added 30 gm. of dry silver oxide in six five-gram lots at intervals of 30 min. The temperature of the reaction mixture was kept at 0–5° C. during the first two additions. It was then raised to 45–50° C. so that the methyl iodide refluxed gently. The procedure thereafter was exactly as described previously (2). The product was treated with a further quantity of 30 gm. of silver oxide and 50 gm. of methyl iodide. It was then purified by dissolving in benzene and precipitating with petroleum ether (b.p. 60–70° C.). This was repeated twice, using, however, petroleum ether (b.p. 30–50° C.) in both cases. Yield, 4 gm. A sample was dried at 80° C. over phosphorus pentoxide at 8 mm. Analysis: 19.7 mg. gave 65.4 mg. AgI; —OCH₃, 44.8%. Calcd. —OCH₃ = 45.6%. $[\alpha]_D^{25} = -89.0^\circ$ in tetrachlorethane ($c = 2.85$).

Hydrolysis of Fully Methylated Raffinose Levan

The procedure followed exactly the description already given by Hibbert and Brauns (2). From 4 gm. of the methylated levan about 1.5 gm. of crystalline 1:3:4 trimethyl fructose was isolated from a light-brownish gum which still continued to show signs of crystallization. M.p. 74° C.; mixed m.p. with authentic specimen, 74° C. Rotation $[\alpha]_D^{25} = +22^\circ$ ($c = 3.82$).

TABLE I
PROPERTIES OF SUCROSE LEVAN, RAFFINOSE LEVAN AND DERIVATIVES

	Sucrose levan	Raffinose levan
	Properties	
Levan	$[\alpha]_D^{22} = -46.1^\circ$	$[\alpha]_D^{22} = -45.5^\circ$
Levan triacetate	$[\alpha]_D^{24} = +9.0^\circ$ (c = 1.96)	$[\alpha]_D^{20} = +8.9^\circ$ (c = 3.01)
Trimethyl levan	$[\alpha]_D^{22} = -88.0^\circ$	$[\alpha]_D^{23} = -89.0^\circ$
Trimethyl fructofuranose	M.p. 74° C. $[\alpha]_D^{22} = +21.55^\circ$ (c = 3.56)	M.p. 74° C. $[\alpha]_D^{25} = +22^\circ$ (c = 3.82)

Acknowledgment

The authors express their indebtedness to Miss Frances Fowler for the preparation of the crude raffinose levan.

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THE SORPTION OF TANNIC ACID BY PROTEINS AND THE MECHANISM OF VEGETABLE TANNING¹

BY WILFRED GALLAY²

Abstract

Tannic acid is taken up by gelatin and casein from aqueous solution according to the adsorption isotherm. The temperature coefficient of this sorption is negative. From aqueous alcohol and aqueous acetone solution, the sorption obeys Henry's law. The sorption of tannic acid by hide powder was measured together with the swelling of the latter and the two found to vary in a parallel manner. The same relation was found for the sorption of tannic acid by hide powder with varying alkalinity. The sorption of sodium hydroxide on hide powder obeys the adsorption isotherm. When various acids are used to adjust the system to constant pH, the sorption of tannic acid on hide powder varies essentially as the degree of swelling of the adsorbent. Relations are shown between the viscosities of ethyl alcohol-water mixtures and the sorption of tannic acid on hide powder from corresponding solutions. No lyotropic effect is obtained upon the addition of electrolytes to the sorption system tannin-gelatin. The results obtained are discussed from the point of view of the mechanism of vegetable tanning, and it is concluded that the amount of sorption depends to a great extent upon the degree of swelling of the adsorbent.

In early investigations of the fixation of tannic acid and tannins by proteins the results were considered from the point of view of the law of definite proportions and numerous attempts were made to determine the relative combining weights of tannin and gelatin. No satisfactory explanation of the widely divergent results obtained could be made, and more recent knowledge of the behavior of colloidal solutions renders the idea of direct compound formation between tannin and protein entirely untenable.

Among the several theories and their modifications with respect to the mechanism of tannin fixation, that due to Procter and Wilson has received the most consideration. According to this theory, the protein must first combine with hydrogen ion, thus becoming positively charged, and that it then, upon coming in contact with tannin particles, combines with them through mutual discharge. According to this conception the hydrogen ion concentration is the only important variable factor in the system.

There are several fundamental objections to this theory. There is some doubt whether tannin bears a charge since some electrophoretic measurements have yielded negative results (4). The fact that no coagulation is brought about by tannin in an isoelectric gelatin sol is readily understood from other considerations. It has been amply shown that the stability of a hydrophilic sol depends upon two factors, *viz.*, charge and hydration, of which the latter is by far the more important. The addition of a tannin sol to an isoelectric gelatin sol changes the character of the latter from hydrophilic to hydrophobic, desolvation being effected and the residual stability being now due solely to the electrokinetic potential. The level of the latter may be readily reduced to the critical one by the addition of traces of electrolytes, when precipitation

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Contribution from the National Research Laboratories, Ottawa, Canada.

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immediately occurs (5). Tannic acid fixation on protein in alkaline medium, although much less than in acid, is nevertheless appreciable and this fact is obviously in direct opposition to a theory involving mutual discharge. Wilson (13, pp. 577-578) offers the explanation that this is due to traces of impurities in the tannic acid and to products formed by hydrolysis and oxidation of the tannic acid.

The amount of tannic acid fixed by protein is known to vary with the concentration and in several cases it has been shown that the relation may be expressed by the empirical general parabolic equation (in this connection commonly termed the adsorption isotherm),

$$\frac{x}{m} = \beta C^{\frac{1}{n}},$$

where $\frac{x}{m}$ is the amount adsorbed per unit weight of adsorbent, C is the concentration of adsorbate at equilibrium, and β and $\frac{1}{n}$ are constants. This expresses a condensation of tannin particles on the surface of the adsorbent. The colloidal units of the latter may be looked upon as being agglomerations of condensed and polymeric chains, loosely bound and forming very porous

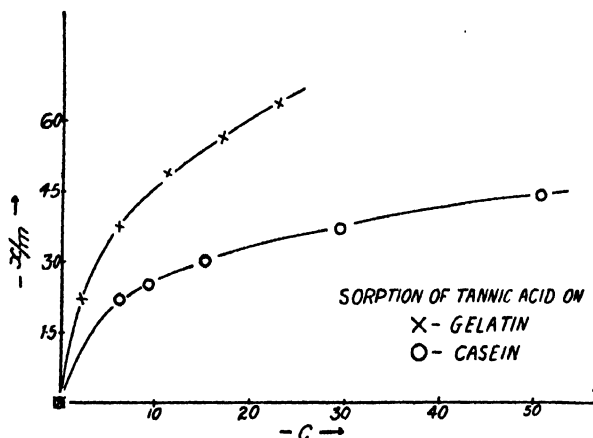


FIG. 1. Sorption of tannic acid on gelatin and casein.

a dissolved substance on it. Whether, however, it possesses this power or not, particles dissolved in the medium, if sufficiently small, will be present within the pores of the micelle as well as in the surrounding medium. There will, then, be two phases, one protein-tannin-water and the other tannin-water. In other words, assuming that there takes place no surface adsorption, there will result a simple equilibrium, a partition of the solute between the protein and the water.

Tables VIII and IX and Fig. 1 show the results obtained in the sorption of tannic acid on gelatin and casein, the adsorbents being in aqueous solution before the addition of the adsorbate.

micelles. The constituent fibres of these micelles have been forced apart by imbibition of the solvent. It is difficult to speak of a surface here in the ordinary sense of the word. The outer surface of the micelle forms probably a very small part of the total effective surface. According to the prevailing conditions, the type of adsorbate and of dispersion medium, this surface may or may not have the power of adsorbing

In Fig. 2 the relation between the amount adsorbed and the concentration is logarithmically shown according to

$$\log \frac{x}{m} = \log \beta + \frac{1}{n} \log C.$$

The values of $\frac{1}{n}$ may be directly measured and the mean values of β calculated.

From these figures there can then be obtained calculated values of $\frac{x}{m}$. In

Tables I and II these values of $\frac{x}{m}$ are compared with the values obtained

experimentally. It is seen that the differences are comparatively small and hence the adsorption isotherm is well obeyed over the range measured.

Cases of pure adsorption are known. As examples may be cited the adsorption of gold and arsenic trisulphide by carbon and barium sulphate (12), and of carbon suspension by paper (8). However in several instances,

investigators have found that adsorption and absorption (solution) occur together. McBain (6) states that hydrogen is partly dissolved and partly

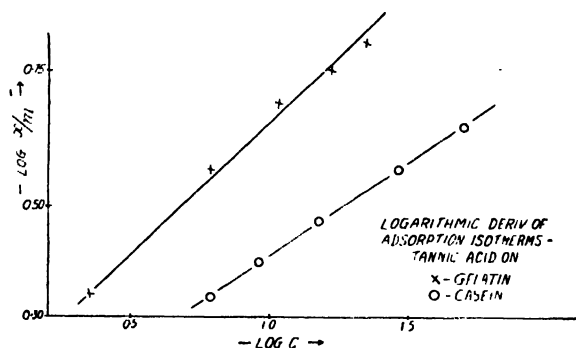


FIG. 2. Logarithmic derivatives of adsorption isotherms-tannic acid on gelatin and casein.

TABLE I

SORPTION OF TANNIC ACID BY GELATIN FROM AQUEOUS SOLUTION

$$\frac{1}{n} = 0.448 \quad \beta = 1.603$$

C, millimoles per litre	22.96	16.98	11.09	6.15	2.24
$\frac{x}{m}$, calc.	6.52	5.70	4.69	3.62	2.30
$\frac{x}{m}$, obs.	6.35	5.65	4.90	3.75	2.20

TABLE II

SORPTION OF TANNIC ACID BY CASEIN FROM AQUEOUS SOLUTION

$$\frac{1}{n} = 0.335 \quad \beta = 1.189$$

C, millimoles per litre	51.06	29.56	15.14	9.28	6.20
$\frac{x}{m}$, calc.	4.45	3.70	2.82	2.49	2.19
$\frac{x}{m}$, obs.	4.43	3.71	2.98	2.50	2.18

adsorbed by charcoal. Georgievics (3) came to the same conclusion with dilute acids and charcoal. Davis (1) and McBain (7) found in the sorption of iodine by charcoal that an initial fast adsorption was followed by slow diffusion and solution over a period of years. It is therefore a difficult matter

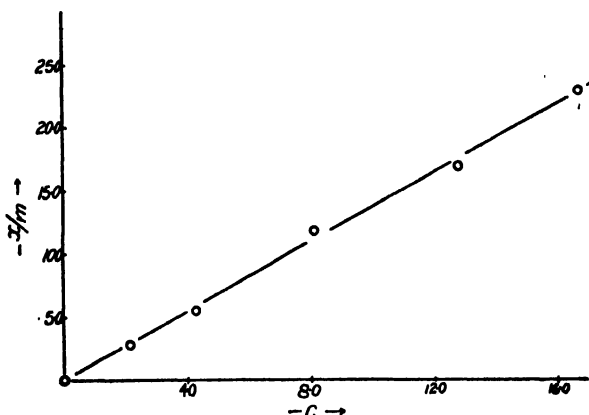


FIG. 3. Sorption of tannic acid on gelatin in ethyl alcohol-water.

then being that of Henry's law,

$$\frac{x}{m} = \beta C.$$

The writer has found this to be true for the case of the sorption of iodine by polyvinyl alcohol (2). This sorption obeys the adsorption isotherm over wide ranges in aqueous solution, but in aqueous alcohol solution, e.g., 75% alcohol, the relation between amount taken up and concentration is expressed by a straight line, a simple partition of the solute between solvent and absorbent. The same has been found in the present instance. The sorptions of tannic acid on gelatin in aqueous alcohol and aqueous acetone solutions obey Henry's law as shown in Tables X and XI and Fig. 3.

The sorptions of tannic acid on gelatin in aqueous alcohol and aqueous acetone solutions obey Henry's law as shown in Tables X and XI and Fig. 3.

The above experiments were carried out at room temperature since it was found that small variations in temperature were without appreciable effect on the extent of sorption. In order to measure the temperature coefficient over a wide range, the sorption of tannic acid on gelatin was carried out at 60°, 45° and 1.5° C. The results are shown in Table XII and Fig. 4.

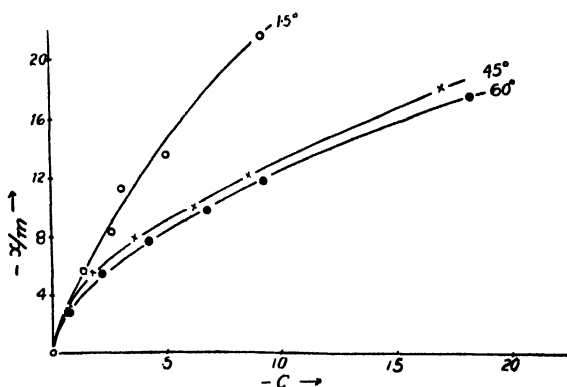


FIG. 4. Sorption of tannic acid by gelatin.

It will be seen that the temperature coefficient is very appreciable over wide ranges and is negative as in the case of charcoal. The curves measured at 60° and 45° C. are affine ($-\frac{1}{n} = 0.560$ and 0.572 respectively), *i.e.*, one is obtained

by multiplying the ordinates of the other by a constant factor. From this may be concluded that the true adsorptive power is the same in each case but the specific surface is greater at the lower temperature. On this basis some change in the nature of the surface has taken place at 1.5° C. causing a marked increase in $\frac{1}{n}$. These differences are particularly striking in the logarithmic derivatives shown in Fig. 5.

In Tables III, IV and V are shown the calculated

and observed values of x , based on the measured $\frac{1}{n}$ and calculated mean β .

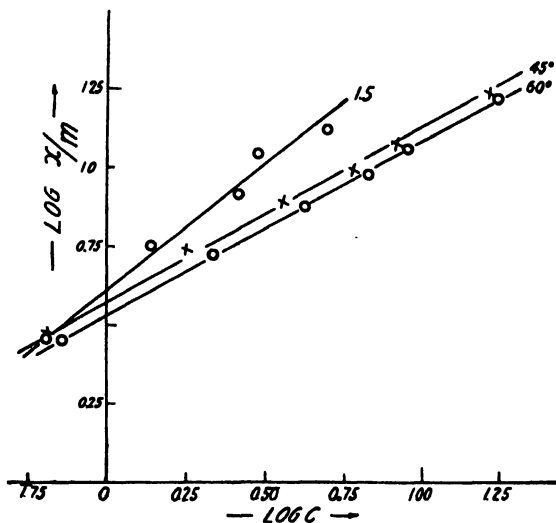


FIG. 5. Logarithmic derivatives of sorption isotherms—tannic acid on gelatin.

TABLE III

SORPTION OF TANNIC ACID ON GELATIN AT 60° C. (AQUEOUS SOLUTION)

$$\frac{1}{n} = 0.560 \quad \beta = 3.405$$

C , millimoles per litre	18.13	9.20	6.75	4.23	2.18	0.73
$\frac{x}{m}$, calc.	17.25	11.80	9.92	7.63	5.27	2.85
$\frac{x}{m}$, obs.	17.58	11.84	9.72	7.62	5.34	2.82

TABLE IV

SORPTION OF TANNIC ACID ON GELATIN AT 45° C. (AQUEOUS SOLUTION)

$$\frac{1}{n} = 0.572 \quad \beta = 3.671$$

C , millimoles per litre	16.90	8.53	6.20	3.60	1.78	0.65
$\frac{x}{m}$, calc.	18.50	12.51	10.42	7.64	5.10	2.87
$\frac{x}{m}$, obs.	18.07	12.11	9.94	7.87	5.50	2.85

TABLE V

SORPTION OF TANNIC ACID ON GELATIN AT 1.5° C. (AQUEOUS SOLUTION)

$$\frac{1}{n} = 0.885 \quad \beta = 0.6287$$

C, millimoles per litre	4.98	3.00	2.60	1.38	0.65
$\frac{x}{m}$, calc.	17.60	11.22	9.91	5.63	2.90
$\frac{x}{m}$, obs.	13.53	11.23	8.29	5.66	2.85

The variation in the amount of tannic acid fixed by collagen has been put forth as a basic point of proof for the Procter-Wilson theory (10, 13, Chap. 16). A minimum of tannage is obtained at the isoelectric point of collagen, the amount fixed increasing sharply on the acid side and slowly on the alkaline side. This is interpreted to mean that fixation on the acid side increases due to increased positive charge on the collagen with consequent greater ability to fix tannin through mutual discharge. This matter may be considered, however, from the point of view of specific surface available for sorption. It is well known that various colloidal properties of proteins show a minimum at the

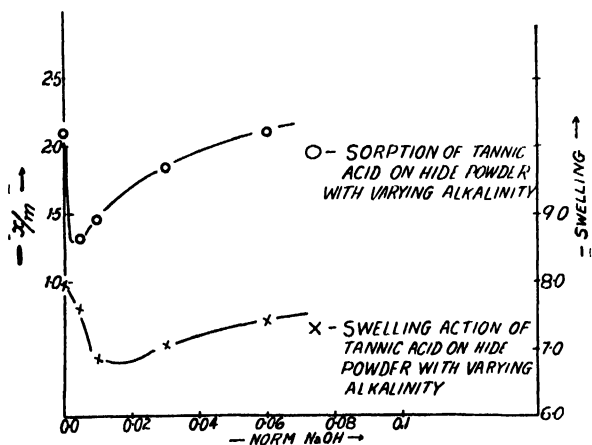


FIG. 6. Sorption and swelling action of tannic acid on hide powder with varying alkalinity.

fixation with pH, without regard to a theory involving mutual discharge.

In Table XIII and in Fig. 6 is shown the variation of tannic acid fixation on hide powder with varying pH and constant concentration of solute, and the swelling of each adsorbent at equilibrium. The similarity is striking, the tannic acid fixation appearing to depend directly upon the degree of solvation of the adsorbent.

Table XIV and Fig. 7 show the variation of sodium hydroxide fixation by hide powder with varying concentration. The adsorption isotherm is fairly well obeyed within the range measured as shown by the logarithmic plot of

isoelectric point, among them being the ability to imbibe solvent. Swelling increases to a maximum with increasing acidity and then decreases, probably due to the dehydrating action of the concentrated acid in the solvent. Since the amount of tannin fixation must be dependent to a great extent upon the specific surface of the protein both for adsorption and solution, a reasonable explanation is at hand for the variation of tannin

Fig. 7 and Table VI, where the observed and calculated values of $\frac{x}{m}$ may be compared.

TABLE VI
SORPTION OF SODIUM HYDROXIDE ON HIDE POWDER
 $\frac{1}{n} = 0.210 \quad \beta = 0.271$

C, millimoles per litre	101.64	57.23	24.36	3.63	0.53
$\frac{x}{m}$, calc.	0.716	0.635	0.531	0.355	0.237
$\frac{x}{m}$, obs.	0.735	0.629	0.527	0.376	0.215

The sorption of tannic acid on hide powder with increasing concentration of adsorbate follows a different path to those in the cases of gelatin and casein in solution. There is a steep rise (Fig. 8) in the amount taken up until a maximum is reached at an intermediate concentration of tannic acid, followed by a decrease. One explanation given for this course is that with increasing concentration of tannic acid, the rate of combination of the latter with collagen increases so rapidly that soon a point is reached where the surfaces of the protein fibres are so heavily tanned that their permeability is reduced to the remainder of the tannic acid in solution. Another explanation is that of Thomas and Foster (9), who observed that the electrical difference of potential at the surface of tannin

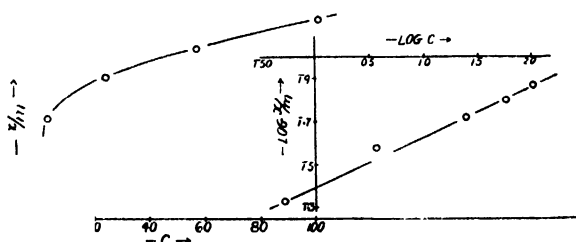


FIG. 7. Sorption of sodium hydroxide on hide powder.

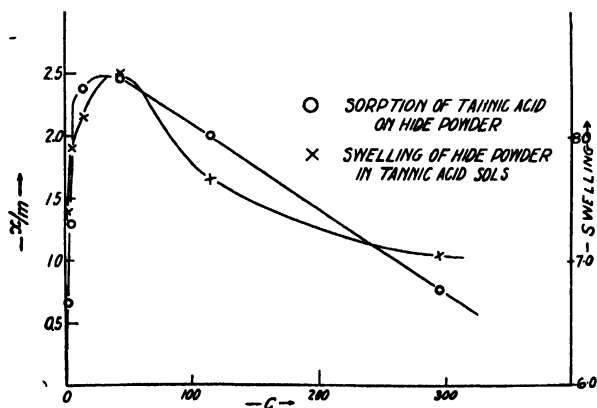


FIG. 8. Sorption of tannic acid on hide powder and swelling of hide powder in tannic acid sols.

particles decreases with increasing concentration of tan liquor. This latter explanation is the one favored by Wilson (13, p. 519), as fitting in with his theory of the mechanism of vegetable tanning. According to this theory, this decrease in potential would lessen the attraction between the tannin particles and the protein gel and thus cause a decrease in the rate of combination.

Table XV and Fig. 8 show the sorption data for tannic acid and hide powder and the corresponding values for the swelling of the adsorbent at equilibrium. As in the case of the series with varying pH discussed above, the fixation of the tannic acid runs parallel to the degree of solvation of the adsorbent.

It must follow directly from the Procter-Wilson theory that at the same pH, no matter how the latter is fixed, the sorption of tannic acid must be constant for the same concentrations of tan liquor. Experiments of Thomas and Kelly (10) however, point to entirely different results. In two parallel sets of experiments in the variation of sorption with concentration of tan liquor, the pH was kept constant at 2 by the addition of acid. In the one case the acid used was hydrochloric and in the other, phosphoric. The fixation in the latter series was by far the greater at equivalent concentrations. At high concentrations of solute the difference amounted to over 100%. The explanation put forth by Wilson (13, p. 574) is that phosphoric acid is a better buffer than hydrochloric, permitting a smaller rise in pH value during tanning or possibly that the increased amount of the weaker acid used to produce the desired pH is responsible. It has been shown that the dissociation constant of the acid used has a great effect on the amount of tannic acid fixed by hide substance (11). The increasing effect of the various acids upon the fixation is essentially the inverse order of the acids arranged according to decreasing dissociation constants. In the present instance a number of sorptions of tannic acid on hide powder at constant pH were measured, the only variation being in the acid used to adjust the system to the desired pH. The swelling of the adsorbent was measured also at equilibrium and the results expressed in Table VII.

TABLE VII
SORPTION OF TANNIC ACID ON HIDE POWDER

Acid used to adjust pH	$\frac{x}{m}$, millimoles per gm.	Swelling of hide powder, per gm.	Acid used to adjust pH	$\frac{x}{m}$, millimoles per gm.	Swelling of hide powder, per gm.
Acetic	2.37	10.1	Oxalic	2.04	7.8
Lactic	2.29	10.2	Tartaric	2.03	9.5
Citric	2.23	9.6	Hydrochloric	1.98	8.4
Phosphoric	2.04	9.9	Sulphuric	1.86	6.6

NOTE: pH, 2.5; total concentration of tannic acid, 2%.

The accuracy of the method used for the swelling measurements is comparatively low. However it may be concluded from the general trend of the swelling of the hide powder that the sorption is again shown to be closely related to the degree of swelling of the adsorbent.

A rather significant conclusion may be drawn from the following relations. There is practically no fixation of tannin on hide powder from alcoholic solution. It would be expected then starting with aqueous solution that with solutions of tannic acid in media containing increasingly large proportions of alcohol, there would be obtained a regular decrease of fixation. This however is not found

to be the case (Fig. 9). There appears a well-marked point of change at an alcoholic concentration of about 50% by volume. It is a striking fact that approximately at this concentration does the viscosity-concentration curve of an alcohol-water system show a maximum. The latter is interpreted to mean that compound formation takes place, the relative proportions of alcohol and water in the compound being given by the position of the maximum. On this basis as the percentage of alcohol in the dispersion medium is gradually increased, water is "bound" by the alcohol resulting in a decreased degree of swelling of the hide powder with a corresponding decrease in tannin fixation. Until the point of maximum compound formation is reached, this decrease is rapid and regular. At this point however, that amount of alcohol necessary to "bind" a maximum of water has been reached and the effect of still greater proportion of alcohol in the dispersion medium is simply that due to a decrease in the amount of water necessary for full imbibition by the adsorbent. The decrease after the intermediate point of change is regular but less rapid than the first decrease. Tables XVII and XVIII and Fig. 9 show the results obtained in this sorption series.

The relative effect of equimolecular concentrations of various electrolytes upon the fixation of tannic acid by gelatin was investigated and the results are shown in Table XIX. The additions of electrolyte were made before precipitation in the first series and after precipitation in the second. Appreciable variations in tannin fixation were obtained but in neither series was there observed any lyotropic effects.

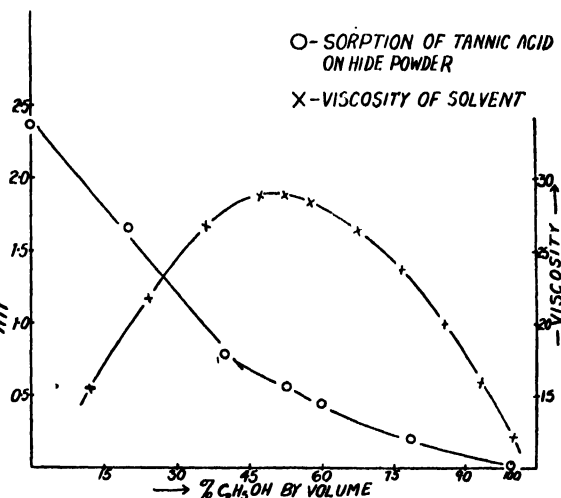


FIG. 9. Sorption of tannic acid on hide powder and viscosity of solvent.

Experimental

The tannic acid used throughout the work was "Tannic Acid puriss", of Schuchardt, and solutions were used immediately after preparation. The gelatin sols were not prepared in a standard way and hence, to some extent, only inter-comparisons of results may be made. The hide powder used was the standard material in use in tannin analysis. The Löwenthal oxidation method was used in tannic acid determinations, the procedure being as follows. Standard indigo carmine solution was prepared by dissolving 5 gm. of the dye in 50 cc. of concentrated sulphuric acid and then diluting to one litre with

distilled water and filtering. Potassium permanganate solutions of concentration 0.5 gm. per litre were used, freshly prepared owing to the poor keeping quality of solutions of this strength. To 25 cc. of the dye solution was added 750 cc. of distilled water and an aliquot part of the tannin sol under investigation. The solution was mechanically vigorously stirred during titration with the permanganate. For dilute tannin sols the end-point, denoted by a change to lemon-yellow from blue-green, was fairly sharp, check experiments agreeing within 1%. The dye was standardized alone in terms of the potassium permanganate solution and then tannic acid standardized by using a solution of known concentration. In order to convert to molar concentrations from the weights thus obtained, tannic acid was calculated as $C_{14}H_{10}O_9$.

The amount adsorbed per unit weight of adsorbent, $\frac{x}{m}$, is expressed as millimoles per gram, and C , the concentration of solute at equilibrium, as millimoles per litre. The exponent of the adsorption isotherm, $\frac{1}{n}$, was obtained by plotting $\frac{x}{m}$ and C on a logarithmic diagram, measuring the angle of inclination between the line joining every two experimental points and the log C axis and taking the mean of the tangents. β was obtained by inserting in each case the value of $\frac{1}{n}$ found, and taking the mean.

To 40 cc. of tannic acid sols of varying concentrations, was added 40 cc. of 0.5% gelatin sol and the mixture shaken mechanically for five hours. The gelatin-tannic acid precipitate was then separated by centrifuging and tannic acid determined in an aliquot part of the supernatant liquor.

TABLE VIII
SORPTION OF TANNIC ACID ON GELATIN FROM AQUEOUS SOLUTION

$$\frac{1}{n} = 0.448 \quad \beta = 1.603$$

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
2.5	1.837	1.269	22.96	6.35
2.0	1.358	1.126	16.98	5.65
1.5	0.887	0.976	11.09	4.90
1.0	0.492	0.750	6.15	3.75
0.5	0.179	0.442	2.24	2.20

In the case of casein, sols of concentration lower than 0.75% left an opalescent solution after precipitation with an equal amount of tannic acid. The procedure was the same as in the case of gelatin.

To 40 cc. of tannic acid sols of varying concentrations was added 40 cc. of 0.5% gelatin sol and the mixtures shaken for one hour. Then 240 cc. of 95% ethyl alcohol was added to each sol. The comparatively highly swollen

precipitates now dehydrated and settled rapidly. The mixtures were shaken mechanically for two hours to equilibrium, and tannic acid determined on an aliquot part of the clear supernatant liquid.

TABLE IX
SORPTION OF TANNIC ACID ON CASEIN FROM AQUEOUS SOLUTION

$$\frac{1}{n} = 0.335 \quad \beta = 1.189$$

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
4.0	4.085	0.885	51.06	4.43
2.5	2.365	0.741	29.56	3.71
1.5	1.268	0.596	15.14	2.98
1.0	0.742	0.500	9.28	2.50
0.75	0.496	0.436	6.20	2.18

TABLE X
PTION OF TANNIC ACID ON GELATIN FROM ALCOHOL-WATER SOLUTION

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
8.0	5.332	4.601	16.66	23.00
6.0	4.069	3.380	12.69	16.90
4.0	2.589	2.378	8.09	11.89
2.0	1.381	1.102	4.31	5.51
1.0	0.687	0.555	2.15	2.78

The above procedure was carried out with acetone in place of alcohol, a longer time being allowed for equilibrium.

TABLE XI
SORPTION OF TANNIC ACID ON GELATIN FROM ACETONE-WATER SOLUTION

Conc. of tannic acid, %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
5.0	5.555	0.653	17.36	3.27
4.0	4.474	0.492	13.98	2.46
3.0	3.385	0.340	10.58	1.70
2.0	2.291	0.192	7.16	0.96
1.0	1.166	0.076	3.64	0.38

To 20 cc. of tannic acid sols of varying concentrations, was added 20 cc. of 0.5% gelatin sol, each at 60° C. The reaction tubes were fitted with capillary outlets and kept in a thermostat at 60° C. for six hours, the contents being stirred from time to time. The mixtures were then filtered through a funnel jacketed by water at 60° C. and tannic acid determined on the filtrates. Two other series were carried out under the same procedure except that the temperatures maintained were 45° and 15° C.

TABLE XII
SORPTION OF TANNIC ACID ON GELATIN AT VARYING TEMPERATURES

	Conc. of tannic acid %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	<i>C</i>	$\frac{x}{m}$
<i>t</i> = 60° C.	4.0	0.725	1.760	18.13	17.58
$\frac{1}{n}$ = 0.560	2.5	0.368	1.187	9.20	11.84
β = 3.405	2.0	0.270	0.973	6.75	9.72
	1.5	0.169	0.764	4.23	7.62
	1.0	0.087	0.534	2.18	5.34
	0.5	0.029	0.282	0.73	2.82
<i>t</i> = 45° C.	4.0	0.676	1.807	16.90	18.07
$\frac{1}{n}$ = 0.572	2.5	0.341	1.211	8.53	12.11
β = 3.671	2.0	0.248	0.994	6.20	9.94
	1.5	0.144	0.787	3.60	7.87
	1.0	0.071	0.550	1.78	5.50
	0.5	0.026	0.285	0.65	2.85
<i>t</i> = 15° C.	2.5	0.199	1.356	4.975	13.53
$\frac{1}{n}$ = 0.885	2.0	0.120	1.123	3.000	11.23
β = 4.253	1.5	0.104	0.829	2.600	8.29
	1.0	0.055	0.566	1.375	5.66
	0.5	0.026	0.285	0.650	2.85

To 100 cc. of 2% tannic acid sols to which varying amounts of sodium hydroxide had been added, were added 2 gm. of hide powder and the mixtures shaken mechanically for five hours and allowed to stand overnight. The mixtures were then filtered, using short stem filter funnels, through cotton which had previously been wet and allowed to drain. All filtrations were made in as standard a way as possible. The receivers were 100-cc. volumetric flasks, and after filtration were filled to the mark with water from a burette, compensating for the water retained by the hide powder. Tannic acid determinations were made in the manner already described. Hydrolysis of the hide substance was marked at the highest concentration of sodium hydroxide.

TABLE XIII
SORPTION OF TANNIC ACID ON HIDE POWDER WITH VARYING ALKALINITY

Sodium hydroxide added, gm.	Tannic acid left in solution, millimoles	Tannic acid fixed, millimoles	$\frac{x}{m}$	Swelling per gm.
0	2.022	4.188	2.09	7.95
0.02	3.576	2.634	1.32	7.60
0.04	3.288	2.922	1.46	6.85
0.12	2.524	3.686	1.84	7.05
0.24	1.987	4.223	2.11	7.40
0.40	—	—	—	—

To 100 cc. of sodium hydroxide solutions of varying concentrations was added 2 gm. of hide powder and after mechanical shaking for five hours the mixtures were centrifuged and sodium hydroxide was determined in the clear liquid.

TABLE XIV
SORPTION OF SODIUM HYDROXIDE ON HIDE POWDER

$$\frac{1}{n} = 0.210 \quad \beta = 0.271$$

Normality of sodium hydroxide	Sodium hydroxide left in soln., millimoles	Sodium hydroxide fixed, millimoles	C	$\frac{x}{m}$
0.1163	10.164	1.470	101.64	0.735
0.0698	5.723	1.257	57.23	0.629
0.0349	2.436	1.054	24.36	0.527
0.0116	0.363	0.751	3.63	0.376
0.0058	0.053	0.429	0.53	0.215

Lots of 2 gm. of hide powder were shaken mechanically for five hours with 100 cc. of tannic acid sols of varying concentrations and then the mixtures allowed to stand overnight. Determinations of the swelling of the hide powder were made in the manner described above and tannic acid was determined in each filtrate.

TABLE XV
SORPTION OF TANNIC ACID ON HIDE POWDER

Conc. of tannic acid %	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$	Swelling per gm.
10.0	29.520	1.540	295.20	0.77	7.05
5.0	11.521	4.009	115.21	2.00	7.65
3.0	4.401	4.917	44.01	2.46	8.50
2.0	1.444	4.768	14.44	2.38	8.15
1.0	0.516	2.590	5.16	1.30	7.90
0.5	0.238	1.315	2.38	0.66	7.40

Table XVI shows the amounts of acids used per litre pre-calculated in order that when diluted to twice the volume with 4% tannic acid solution, the final pH obtained be approximately 2.5. The pH was measured by means of a comparator, methyl violet (range 1.8 to 3.2) serving as indicator in all cases.

TABLE XVI
VARIOUS ACIDS USED AND ADJUSTED pH

Acid	Amount used per litre	pH after addition of tannic acid	Acid	Amount used per litre	pH after addition of tannic acid
Hydrochloric	0.53 cc.	2.5	Oxalic	0.832 gm.	2.4
Sulphuric	0.30 cc.	2.5	Lactic	10.81 cc.	2.4
Phosphoric	0.47 cc.	2.4	Tartaric	4.50 gm.	2.4
Acetic	80.0 cc.	2.4	Citric	6.72 gm.	2.4

Lots of 2 gm. of hide powder were shaken mechanically for five hours with 100 cc. of the above acid-tannic acid mixtures and then allowed to stand one

week, after which swelling and tannic acid determinations were made in the usual manner. The results are expressed in Table VII.

Tannic acid solutions (2%) were prepared in media containing varying proportions of 95% ethyl alcohol. Lots of 2 gm. of hide powder were shaken mechanically for six hours with 100 cc. of these solutions and allowed to stand overnight after which tannic acid determinations were made.

TABLE XVII
SORPTION OF TANNIC ACID ON HIDE POWDER FROM WATER-ALCOHOL SOLUTIONS

Ethyl alcohol, % by volume	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	$\frac{x}{m}$
100	6.154	0.054	0.027
80	5.804	0.404	0.202
60	5.312	0.896	0.448
50	5.054	1.154	0.577
40	4.631	1.577	0.789
20	2.877	3.331	1.666
0	1.473	4.735	2.368

The following relations between water-alcohol percentage by weight and viscosity of the mixture were taken from the International Critical Tables. The percentage by volume has been inserted for purposes of comparison with Table XVII.

TABLE XVIII
VISCOSITY OF WATER-ETHYL ALCOHOL MIXTURES

Ethyl alcohol, % by weight	Ethyl alcohol, % by volume	Viscosity (poises)	Ethyl alcohol, % by weight	Ethyl alcohol, % by volume	Viscosity (poises)
10	12.39	15.5	60	67.69	26.4
20	24.47	21.7	70	76.91	23.7
30	36.18	26.7	80	85.46	20.0
40	47.33	28.7	90	93.25	16.0
45	52.66	28.7	100	100.00	12.2
50	57.83	28.3			

TABLE XIX
EFFECT OF ELECTROLYTES ON TANNIC ACID SORPTION BY GELATIN

Series	Electrolyte	Tannic acid left in soln., millimoles	Tannic acid fixed, millimoles	C	$\frac{x}{m}$
A	NaCl	2.104	1.002	6.58	5.00
	NaBr	2.464	0.642	7.70	3.20
	NaNO ₃	2.415	0.691	7.55	3.45
	Na ₂ SO ₄	2.355	0.751	7.36	3.75
B	NaCl	1.888	1.218	5.90	6.10
	NaBr	1.965	1.141	6.14	5.70
	NaNO ₃	1.926	1.181	6.02	5.90
	Na ₂ SO ₄	2.003	1.103	6.26	5.50

A. Gelatin sol (40 cc. of 0.5%) was added to 40 cc. of 2.5% tannic acid sol in each of four flasks and after mechanical shaking for two hours, there was added to each 240 cc. of inorganic salt solutions of such concentration, that the salt was present in molar solution in the whole mixture. After five hours of further shaking, the mixtures were centrifuged and tannic acid determinations made.

B. A second series was then carried out with the same procedure except that the electrolyte was added to the gelatin sol before precipitation by tannic acid.

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CHEESE-RIPENING STUDIES¹

Nitrogen Requirements of Lactic Acid Bacteria

I. THE FRACTIONAL ANALYSIS OF VARIOUS NITROGEN SOURCES USED FOR THE QUANTITATIVE DETERMINATION OF THE SUGAR-FERMENTING ABILITIES OF LACTIC ACID BACTERIA

BY BLYTHE ALFRED EAGLES² AND WILFRID SADLER³

Abstract

A study is being made of the nitrogen requirements of lactic acid bacteria. Employing the method of Wasteneys and Borsook, the nitrogen distribution has been determined in nitrogen sources available commercially and in sources that may be readily prepared by laboratory workers. Forty-three sources have been analyzed. The results of the analyses show that peptic casein digest broth contains from 55 to 63% protein nitrogen, 19 to 25% peptone nitrogen, and 14 to 17% subpeptone nitrogen, according to the particular casein used—when the standard method of preparation is followed. If less casein is used for digestion, or if the period of digestion is reduced, the total amount of nitrogen made available is lower; this being true for the subpeptone nitrogen fraction in particular. In tryptic casein digest broth, 70% of the nitrogen is in the subpeptone nitrogen fraction, and about 28% is found as peptone nitrogen. The broth prepared from one commercial source presents a nitrogen distribution picture that is something of a composite of the nitrogen distribution in the standard casein digest and the tryptic casein digest.

When the nitrogen sources fractionated are employed as the substrate for fermentation studies, it will be seen that whilst the suitability of a source is not always fully indicated by the nitrogen distribution picture, the biological significance of the nitrogen distribution in the sources is, on the whole, reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

For the quantitative determination of the sugar-fermenting abilities of the lactic acid bacteria, Orla-Jensen (2) has shown the importance of employing a suitable nitrogen source. His findings have assumed an added significance as the present authors have worked on the bacteria isolated from ripening Kingston cheese. Supplying nitrogen sources comparable with the breakdown products defined in cheese, they have investigated the influence of these sources on the sugar-fermenting abilities of organisms isolated. It was first necessary to determine the nitrogen distribution in nitrogen sources available commercially, and in sources that may be readily prepared by laboratory workers. Where the material has been prepared by the authors they have described precisely the mode of preparation: where the source is of commercial origin the authors have retained the name used for the product by the manufacturer.

For the nitrogen distribution determinations the method of Wasteneys and Borsook has been employed (1, 6).

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Experimental

DEFINITION AND DESCRIPTION OF NITROGEN SOURCES SUBMITTED TO ANALYSIS*

NUMBER

- 1 Peptic casein digest broth prepared after the manner of Orla-Jensen (2, 3) from commercial casein. To 3000 cc. of tap water are added 280 gm. of commercial casein (Will Corporation), 16 gm. of pepsin and 36 cc. of concentrated hydrochloric acid — Orla-Jensen uses 8 gm. of pepsin. With frequent shaking the whole is allowed to digest for 10 days at 37° C. The digest is then filtered, using Munktell's No. 8 filter paper, made up to 2500 cc. with water, and to it are added 10 gm. of dipotassium hydrogen phosphate and 5 gm. of magnesium sulphate. Sufficient 12 *N* sodium hydroxide is added to adjust the broth to a pH of 6.8. NOTE: this broth approximates that from which Orla-Jensen prepares, by dilution with an equal volume of water, the standard casein digest broth used by him in his fermentation studies (2).
- 2 Peptic casein digest broth, prepared by diluting No. 1 with an equal volume of water. NOTE: This broth approximates the standard casein digest broth of Orla-Jensen (2).
- 3 Peptic casein digest broth, prepared in the following manner: to 3000 cc. of tap water are added 140 gm. of commercial casein (Will Corp.), 8 gm. of pepsin and 36 cc. of concentrated hydrochloric acid. With frequent shaking the whole is allowed to digest for one day at 37° C.: subsequent preparation as described for No. 1.
- 4 Peptic casein digest broth: prepared as No. 3 except that digestion continues for two days.
- 5 Peptic casein digest broth: prepared as No. 3 except that digestion continues for 10 days.
- 6 Peptic casein digest broth: prepared as No. 1 but digestion continuing for one day only.
- 7 No. 6 diluted with an equal volume of water.
- 8 Peptic casein digest broth: prepared as No. 1 but digestion continued for two days only.
- 9 No. 8 diluted with an equal volume of water.
- 10 Peptic casein digest broth: prepared as No. 1.
- 11 No. 10 diluted with an equal volume of water.
- 12 Peptic casein digest broth prepared from Vitamine-Free casein (B.D.H.): method of preparation as described for No. 1.
- 13 No. 12 diluted with an equal volume of water.
- 14 Peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.): method of preparation as for No. 1.
- 15 No. 14 diluted with an equal volume of water.
- 16 Peptic casein digest broth prepared from light white soluble casein (B.D.H.): method of preparation as for No. 1.

*After preparation, each respective broth, in quantities not greater than 1500 cc., is sterilized for 25 min. at 12 lb. pressure.

- 17 No. 16 diluted with an equal volume of water.
- 18 Peptic casein digest broth prepared from casein made according to Plimmer (4): method of preparation as for No. 1.
- 19 No. 18 diluted with an equal volume of water.
- 20 Tryptic casein digest broth prepared as follows: to 500 cc. of 1% sodium carbonate solution is added 45 gm. of commercial casein (Will Corp.). The solution is allowed to remain at 37° C. for 24 hr., and 50 cc. of an 87% glycerol extract (10 parts 87% glycerol to 1 part dry gland) of dried pig's pancreas prepared according to the method of Willstätter (7) is then added. Digestion is continued for 10 days, the pH of the digest being adjusted to pH 7.6 from time to time. The digest is then filtered, made up to 420 cc. with water, and to it are added 1.67 gm. of dipotassium hydrogen phosphate and 0.88 gm. of magnesium sulphate. Sufficient 6 *N* hydrochloric acid is then added to adjust the broth to a pH of 6.8.
- 21 No. 20 diluted with an equal volume of water.
- 22 Peptic blood fibrin digest broth prepared as follows: to 375 cc. of tap water are added 35 gm. of blood fibrin*, 2 gm. of pepsin and 4.5 cc. of concentrated hydrochloric acid. With frequent shaking the whole is allowed to digest for six days at 35° C. On the second and third days respectively 0.5 cc. of concentrated hydrochloric acid is added to the digest. The digest is then filtered, made up to 325 cc. with water, and to it are added 1.25 gm. of dipotassium hydrogen phosphate and 0.63 gm. of magnesium sulphate. Sufficient 12 *N* sodium hydroxide is then added to adjust the broth to a pH of 6.8.
- 23 No. 22 diluted with an equal volume of water.
- 24 Peptic blood fibrin digest broth: prepared as No. 22 except that the digestion continues for 10 days.
- 25 No. 24 diluted with an equal volume of water.
- 26 Witte's peptone broth: 69.6 gm. of Witte's peptone, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.
- 27 No. 26 diluted with an equal volume of water.
- 28 Difco proteose peptone broth: 74.0 gm. of proteose peptone (Difco), 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.
- 29 No. 28 diluted with an equal volume of water.
- 30 Hydrolyzed casein (Difco) broth—Bacto tryptophane broth—: 83.2 gm. hydrolyzed casein, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.
- 31 No. 30 diluted with an equal volume of water.

*Blood fibrin was prepared from fibrin as obtained from the abattoir by washing with water for 24 hr., grinding, again washing with water for 24 hr., and then dehydrating by successive treatment with 50, 75, 90 and 95% alcohol. It was then dried at room temperature and ground to a fine powder.

- 32 Bacto-peptone broth: 65.2 gm. Bacto peptone, 4 gm. of dipotassium hydrogen phosphate and 2 gm. of magnesium sulphate dissolved in one litre of water.
- 33 No. 32 diluted with an equal volume of water.
- 34 Bacto yeast extract broth: 150.6 gm. of yeast extract (Bacto) added to approximately 700 cc. of water, heated to 80° C. and filtered. The residue on the filter paper is well washed with warm water, and filtrate and washings cooled and made up to 1000 cc. with water.
- 35 No. 34 diluted with an equal volume of water.
- 36 Marmite broth: 159.4 gm. of marmite made up to one litre with water: method of preparation as described for No. 34.
- 37 No. 36 diluted with an equal volume of water.
- 38 Yeast Vitamine-Harris broth: 1630 yeast Vitamine-Harris tablets made up to one litre with water: method of preparation as described for No. 34.
- 39 No. 38 diluted with an equal volume of water.
- 40 Yeast extract (from Orla-Jensen direct) broth: 161.0 gm. of yeast extract made up to one litre with water: method of preparation as described for No. 34.
- 41 No. 40 diluted with an equal volume of water.
- 42 Bacto peptonized milk broth: 156.9 gm. of peptonized milk (Bacto) dissolved in one litre of water.
- 43 Bacto-gelatin broth: 65.5 gm. of gelatin (Bacto) dissolved in one litre of water.

The results of the determinations of the nitrogen distribution in the sources defined and described are given in Table I.

The results of the analyses show that peptic casein digest broth prepared from commercial casein (Will Corporation), acetic precipitated soluble casein (B.D.H.) or light white soluble casein (B.D.H.) contains from 55 to 63% of proteose nitrogen, 19 to 25% of peptone nitrogen and 14 to 17% of subpeptone nitrogen according to the particular casein used, when the standard method of preparation is followed (2, 3). The distribution of the nitrogen in these three digests is of the same order. If less casein is used for digestion or if the period of digestion is reduced, the total amount of nitrogen made available is lower; this being true for the subpeptone nitrogen fraction in particular.

The peptic digest of blood fibrin contains equal amounts of the proteose nitrogen and peptone nitrogen fractions; and the subpeptone nitrogen fraction is comparable with this fraction of the peptic casein digests.

In the tryptic casein digest broth prepared from commercial casein, 70% of the nitrogen is in the subpeptone nitrogen fraction, and about 28% is found as peptone nitrogen.

Of the commercial sources analyzed, Difco proteose peptone broth and Bacto-peptone broth show a nitrogen distribution of the same order, and dissimilar from the distribution found in any other source analyzed. Both sources contain much more peptone nitrogen, more subpeptone nitrogen and

TABLE I
NITROGEN DISTRIBUTION IN DEFINED NITROGEN SOURCES

Source of nitrogen Number*	Nitrogen in grams per 100 cc. of broth					Per cent of total nitrogen			
	Total nitrogen	Protein nitrogen	Proteose nitrogen	Peptone nitrogen	Sub-peptone nitrogen	Protein nitrogen	Proteose nitrogen	Peptone nitrogen	Sub-peptone nitrogen
1	0.966	0.046	0.556	0.196	0.166	4.76	57.69	20.36	17.19
2	0.483	0.023	0.278	0.098	0.083	4.76	57.69	20.36	17.19
3	0.409	0.059	0.284	0.043	0.023	14.41	69.42	10.40	5.77
4	0.453	0.023	0.326	0.074	0.030	4.99	72.05	16.24	6.72
5	0.494	0.019	0.348	0.067	0.060	3.53	70.52	13.52	12.43
6	0.500	0.054	0.318	0.096	0.032	11.05	63.97	19.05	5.93
7	0.250	0.027	0.159	0.048	0.016	11.05	63.97	19.05	5.93
8	0.724	0.044	0.460	0.160	0.060	5.96	63.57	22.07	8.40
9	0.362	0.022	0.230	0.080	0.030	5.96	63.57	22.07	8.40
10	0.894	0.040	0.598	0.168	0.088	4.41	65.32	18.32	11.95
11	0.447	0.020	0.299	0.084	0.044	4.41	65.32	18.32	11.95
12	0.552	0.0012	0.300	0.154	0.086	0.22	54.28	27.75	17.75
13	0.275	0.0006	0.150	0.077	0.043	0.22	54.28	27.75	17.75
14	0.816	0.0172	0.514	0.160	0.124	2.10	63.04	19.66	15.20
15	0.408	0.0086	0.257	0.080	0.062	2.10	63.04	19.66	15.20
16	0.906	0.044	0.502	0.232	0.128	4.86	55.39	25.66	14.09
17	0.453	0.022	0.251	0.116	0.064	4.86	55.39	25.66	14.09
18	1.032	0.074	0.632	0.228	0.098	7.16	61.29	22.16	9.39
19	0.516	0.037	0.316	0.114	0.049	7.16	61.29	22.16	9.39
20	1.068	0.026	0.0	0.296	0.746	2.50	0.0	27.68	69.82
21	0.534	0.013	0.0	0.148	0.373	2.50	0.0	27.68	69.82
22	0.960	0.054	0.400	0.366	0.140	5.66	41.72	38.17	24.45
23	0.480	0.027	0.200	0.183	0.070	5.66	41.72	38.17	24.45
24	1.176	0.030	0.480	0.464	0.202	2.55	40.78	39.38	17.29
25	0.588	0.015	0.240	0.232	0.101	2.55	40.78	39.38	17.29
26	1.000	0.229	0.464	0.229	0.078	22.88	46.43	22.93	7.76
27	0.500	0.115	0.232	0.114	0.039	22.88	46.43	22.93	7.76
28	1.000	0.0	0.297	0.478	0.225	0.0	29.68	47.84	22.48
29	0.500	0.0	0.149	0.239	0.112	0.0	29.68	47.84	22.48
30	1.000	0.048	0.358	0.273	0.321	4.82	35.84	27.26	32.08
31	0.500	0.024	0.179	0.137	0.165	4.82	35.84	27.26	32.08
32	1.000	0.0	0.215	0.494	0.291	0.0	21.55	49.43	29.02
33	0.500	0.0	0.108	0.247	0.145	0.0	21.55	49.43	29.02
34	1.000	0.0	0.0	0.242	0.758	0.0	0.0	24.20	75.80
35	0.500	0.0	0.0	0.121	0.379	0.0	0.0	24.20	75.80
36	1.000	0.042	0.0	0.394	0.564	4.22	0.0	39.29	56.49
37	0.500	0.021	0.0	0.197	0.282	4.22	0.0	39.29	56.49
38	1.000	0.0	0.0	0.368	0.632	0.0	0.0	36.86	63.14
39	0.500	0.0	0.0	0.184	0.316	0.0	0.0	36.86	63.14
40	1.000	0.022	0.0	0.326	0.652	2.19	0.0	32.59	65.22
41	0.500	0.011	0.0	0.163	0.326	2.19	0.0	32.59	65.22
42	1.000	0.0362	0.059	0.430	0.480	3.62	5.29	43.05	48.04
43	1.000	0.027	0.793	0.156	0.030	2.07	79.31	15.61	3.01

*See foregoing definitions and descriptions.

much less proteose nitrogen respectively than the peptic digests of casein contain. In Witte's peptone the subpeptone nitrogen fraction is little greater than the subpeptone nitrogen fraction in a broth prepared by digesting commercial casein with pepsin for two days only.

The nitrogen distribution in yeast extract (Difco) broth is of the same order as the distribution in the standard tryptic casein digest.

Hydrolyzed casein (Difco) broth presents a nitrogen distribution picture that is something of a composite of the nitrogen distribution in the standard peptic casein digest and the tryptic casein digest.

When the nitrogen sources fractionated are employed as the substrate for fermentation studies, it will be seen that whilst the suitability of a source is not always fully indicated by the nitrogen distribution picture, the biological significance of the nitrogen distribution in the sources is, on the whole, reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

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CHEESE-RIPENING STUDIES¹

Nitrogen Requirements of Lactic Acid Bacteria

II. THE INFLUENCE OF DEFINED NITROGEN SOURCES ON THE SUGAR-FERMENTING ABILITIES OF LACTIC ACID BACTERIA

BY WILFRID SADLER², BLYTHE ALFRED EAGLES³ AND GLADYS PENDRAY⁴

Abstract

The influence of 36 nitrogen sources on the sugar-fermenting abilities of five cultures isolated from cheese has been studied. The five cultures are Gram positive coccus forms that fail to liquefy gelatin: some appear as chains in young milk culture and some are seen as pairs. Three sugars, glucose, mannose and lactose have been used for the work. The fermentation studies have been done after the manner of Orla-Jensen: the suitability of a nitrogen source being interpreted in terms of the total titratable acidity produced by the organisms from defined sugars, after 14 days incubation at the appropriate temperature.

For each organism, peptic casein digest broth is a very suitable source of nitrogen when the standard method of preparation is followed, and the total nitrogen content of the broth is approximately 1%. If the broth be diluted to contain 0.5% total nitrogen, the total titratable acidity obtainable is commonly less by one-third. Containing approximately 1% total nitrogen or 0.5% total nitrogen, tryptic casein digest broth is unsatisfactory as a nitrogen source for cultures EMB₁ 173 and 195; but is very suitable for cultures EMB₂ 166, 168 and 173,—providing the total nitrogen content of the broth is 0.5% rather than 1% total nitrogen.

In the broth prepared from two commercial peptones, the nitrogen distribution is of the same order, but in each case dissimilar from the nitrogen distribution in peptic casein digest or in tryptic casein digest: one peptone broth—1% total nitrogen content—is a very suitable source of nitrogen for all the organisms; but, apart from the fermentation of mannose by two strains, the other peptone broth is no more satisfactory than is a peptic casein digest containing 0.5% nitrogen. In a commercial hydrolyzed casein broth, the nitrogen distribution is something of a composite picture of the distribution in peptic casein digest broth and tryptic casein digest broth. This source is less suitable for culture EMB₁ 173 than is peptic casein digest broth, equally suitable with peptic casein digest for culture EMB₁ 195 and, for cultures EMB₂ 166, 168 and 173, is the best nitrogen source investigated. The hydrolyzed casein broth containing 1% total nitrogen is much more suitable for each culture than is the same broth diluted to contain 0.5% total nitrogen.

Differentiation as between cultures EMB₁ 173 and 195, on the one hand, and cultures EMB₂ 166, 168 and 173, on the other hand, may be obtained by employing certain of the nitrogen sources investigated.

It has been shown that, when the nitrogen sources fractionated are employed as the substrate for fermentation studies, the suitability of a source is not necessarily indicated by the nitrogen distribution picture: even so, it is to be seen that, on the whole, the biological significance of the nitrogen distribution in a source is reflected in the influence on the sugar-fermenting abilities of the lactic acid bacteria reported upon.

The results of the fermentation study show clearly that if the "kind" of nitrogen made available is suitable, the "amount" of nitrogen supplied is then equally important.

For this study the authors chose five cultures on which they had been working for over a year. Cultures EMB₁ 173 and 195 were isolated from a Kingston cheese of the "make" of December 4, 1929—at the time the cheese

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was two weeks old—and are two of some 365 strains then recovered. Cultures EMB₁ 166, 168 and 173 were isolated from another Kingston cheese of the “make” of December 4, 1929, at the time the cheese was two months old. The five cultures are Gram positive coccus forms and fail to liquefy gelatin: some appear as chains in young milk culture and some are to be seen as pairs: the total titratable acidity produced by each strain in milk and in milk enriched with yeast extract has been determined repeatedly (6). The casein-splitting abilities of the organisms have been determined (2) and the work repeated some months later, and the effect on their sugar-fermenting abilities of enriching the nitrogen source with yeast extract has been studied (5).

Experimental

Employing each of 36 nitrogen sources (1), as the substrate, the authors prepared glucose, mannose and lactose broth respectively.

In each case the respective sugar is added at the rate of 2%. The broth is then tubed and plugged—10 cc. in each test tube—and is sterilized at 12 lb. pressure for 20 min. After sterilization each tube is inoculated with the desired culture. Uniformly a 2-mm. loop inoculation from a vigorous growth in milk or casein digest broth is made—milk or broth used for this purpose being enriched with a trace of yeast extract. After inoculation, the series with controls are incubated for 14 days at the appropriate temperature—in this case 23°C. When incubation is completed, the cultures are titrated with *N*/4 sodium hydroxide using phenolphthalein as indicator; the titration of the controls deducted and the results worked out and recorded as grams lactic acid per mille. It can be seen, that in the preparing of a sugar broth and in the manner of incubating, titrating, and recording of results the authors follow the procedure employed by Orla-Jensen (1, 3, 4).

The results of the determinations of the total titratable acidity produced by each of the five cultures are given in Table I.

Discussion

The total titratable acidity produced from each of the three sugars shows that when the total nitrogen content of the broth is approximately 1%, the peptic casein digest prepared from commercial casein (Will Corporation), source No. 1, is a very suitable source of nitrogen for all the cultures. When the same broth is diluted so that the total nitrogen content is approximately 0.5%, source No. 2, this source is not unsuitable in terms of “kind” of nitrogen; but the amount of acid produced by each organism from the respective sugars is reduced to the extent of about one-third, Table I (see also (1), Table I). The titration figures indicate that if the “kind” of nitrogen available is suitable, the “amount” of nitrogen supplied is equally important—interpreting the suitability of a nitrogen source in terms of the total titratable acidity produced by the organisms from the sugars submitted to fermentation.

The effect of departing from the usual method of preparing a peptic casein digest broth (1, 3, 4), may be seen in the nitrogen distribution in sources

TABLE I
THE INFLUENCE OF DEFINED NITROGEN SOURCES ON THE SUGAR-FERMENTING ABILITIES OF LACTIC ACID BACTERIA

Nitrogen source* Number	Culture EMB ₁ 173			Culture EMB ₁ 195			Culture EMB ₂ 166			Culture EMB ₂ 168			Culture EMB ₂ 173		
	Dex-trose	Man-nose	Lac-tose	Dex-trose	Man-nose	Lac-tose	Dex-trose	Man-nose	Lac-tose	Dex-trose	Man-nose	Lac-tose	Dex-trose	Man-nose	Lac-tose
1	7.2	7.7	8.3	6.8	7.0	6.5	6.3	6.8	7.4	6.1	6.3	7.7	5.2	6.3	7.7
2	4.5	4.5	4.5	4.1	4.5	4.7	3.6	4.3	5.0	3.6	4.1	4.7	3.2	2.9	4.5
3	5.2	5.0	4.3	4.7	4.7	3.8	3.2	2.3	2.5	3.4	2.7	2.3	3.2	2.5	2.7
4	5.2	5.0	4.7	5.0	5.4	3.8	4.3	2.9	3.8	4.7	3.4	3.6	5.4	4.1	5.0
5	6.1	5.9	5.6	5.6	5.4	5.4	3.2	2.7	3.6	3.4	2.0	3.2	4.1	2.5	3.6
6	5.0	5.4	4.7	5.6	3.9	5.2	3.2	1.8	3.8	1.8	1.8	1.6	3.8	3.2	4.5
7	3.4	3.2	2.9	3.6	3.4	3.2	2.0	2.7	1.8	4.7	3.8	4.3	2.0	1.8	2.0
8	5.9	5.9	5.2	5.6	5.4	5.2	4.1	3.2	4.3	2.5	1.8	2.3	5.6	3.6	5.4
9	3.6	3.4	3.4	3.4	3.4	3.4	2.3	1.8	2.0	2.5	1.8	2.3	2.3	1.6	2.3
10	7.2	5.9	6.3	7.0	3.6	5.9	5.6	4.3	4.7	5.9	4.3	5.9	7.7	5.6	7.7
11	4.1	3.8	4.1	3.6	3.6	3.8	4.1	2.9	3.8	3.4	3.2	3.6	3.8	3.2	4.5
12	4.1	3.2	3.4	5.6	4.3	3.6	4.1	5.4	3.6	4.3	6.8	3.8	2.3	5.9	2.5
13	4.1	3.2	2.9	3.8	3.8	2.3	2.0	3.8	2.5	2.3	4.3	2.3	2.3	4.1	1.8
14	6.5	6.5	5.4	7.9	6.1	6.1	8.1	9.9	7.2	8.3	9.0	7.0	7.4	8.6	6.1
15	4.3	3.8	3.6	4.7	4.3	4.1	5.4	8.6	4.5	5.9	6.8	4.5	5.0	6.1	3.2
16	7.7	7.2	7.0	7.4	6.8	6.5	7.2	7.4	7.4	7.7	8.1	7.2	7.7	9.0	7.7
17	4.7	4.3	4.3	4.1	3.6	4.1	4.5	5.6	4.7	4.7	5.9	4.5	4.7	5.0	4.3
19	4.7	4.3	4.3	4.1	3.6	3.8	2.3	1.6	1.8	1.8	1.8	1.6	2.9	1.8	1.8
20	0.7	0.7	1.4	0.9	0.7	2.7	4.5	5.6	4.7	2.5	7.2	5.9	2.3	2.5	2.9
21	2.5	1.6	2.7	2.7	2.3	3.2	8.3	7.4	6.5	7.4	7.2	5.9	8.3	8.6	2.9
22	7.4	4.3	2.5	6.3	3.4	3.6	3.8	3.2	2.7	4.3	2.9	2.5	4.7	3.8	3.4
23	4.7	4.3	2.5	8.1	8.1	4.3	4.3	2.3	2.7	4.1	2.5	2.0	7.2	4.1	3.2
24	0.5	5.0	5.0	5.2	5.0	4.3	2.7	2.3	2.5	2.0	1.1	3.8	4.5	1.6	3.8
25	5.6	6.3	6.3	7.0	7.0	6.3	2.3	2.7	3.8	2.7	1.1	2.0	1.8	1.4	2.0
26	6.8	3.4	3.6	3.8	4.7	3.8	1.6	1.6	2.0	1.8	2.0	6.1	1.1	9.0	7.4
27	3.8	7.7	5.9	8.1	7.4	6.3	6.5	6.1	6.3	6.8	8.1	6.3	7.9	7.7	5.9
28	8.1	4.7	3.8	5.0	4.5	3.2	4.5	10.6	4.3	9.0	5.9	11.5	11.0	11.7	12.2
29	5.2	4.7	3.8	7.0	7.4	7.2	10.4	8.3	11.5	9.0	10.1	11.5	11.0	11.7	12.2
30	6.5	6.3	6.5	7.0	7.4	7.2	7.7	10.6	8.1	7.4	8.1	8.1	7.4	9.0	8.8
31	4.5	4.5	4.3	4.3	4.3	4.1	7.7	8.3	8.1	7.4	8.1	8.1	7.4	9.0	8.8
32	3.2	2.9	2.7	4.5	4.1	2.5	3.6	6.5	3.6	4.1	5.6	4.7	3.8	2.9	5.0
33	3.2	2.9	2.7	2.9	2.9	4.5	1.8	5.4	2.0	2.0	6.1	2.5	2.3	6.1	2.9
34	0.0	0.0	2.0	2.5	1.4	1.8	5.2	6.5	3.4	5.4	7.0	4.1	6.5	8.1	4.3
35	2.7	1.6	0.0	3.6	2.9	2.0	3.8	6.5	3.8	3.6	6.1	3.6	4.7	6.8	4.1
36	0.7	0.0	0.5	0.9	1.1	1.8	0.5	0.7	0.7	0.5	1.1	0.9	0.9	0.9	1.6
37	1.8	1.1	0.9	1.8	2.3	1.1	2.3	2.7	2.3	2.7	2.9	2.3	2.7	5.4	1.6

NOTE.—Results recorded as grams lactic acid per millie.
* For descriptions and analyses of nitrogen sources see paper by Eagles and Sadler (1).

Nos. 3, 4, 5, 6 and 8 (1); and in the influence of the sources on the sugar-fermenting abilities of the organisms, Table I. It is to be seen that whilst the subpeptone nitrogen fraction in source No. 5 is low, this fraction is very low in sources Nos. 3, 4, 6 and 8 (1); and there would appear to be a relation of the order of the nitrogen distribution to the low titratable acidity produced by cultures EMB₂ 166, 168 and 173 from each of the three sugars, Table I. Even for cultures EMB₁ 173 and 195 these digests cannot compare as nitrogen sources with the casein digest broth supplied as sources Nos. 1, 14 or 16, Table I.

According to the method of preparation, the peptic casein digest broth source No. 10 should be closely comparable with source No. 1 (1). The nitrogen breakdown in the former however is not as complete as in the latter: much less subpeptone nitrogen is made available (1), and the source is less suitable for cultures EMB₂ 166 and 168, Table I. On the other hand, for the fermentation of dextrose by cultures EMB₁ 173 and 195, and the fermentation of dextrose and lactose by culture EMB₂ 173, the nitrogen supplied as source No. 2 is satisfactory, Table I. By comparing the influence of nitrogen source No. 10 and the diluted broth No. 11 on the amount of acid produced by the organisms, the importance of "amount" as well as "kind" of nitrogen supplied is clearly shown, Table I.

Both the peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.) and light white soluble casein (B.D.H.), sources Nos. 14 and 16 respectively (1), are very good sources of nitrogen for the five organisms; and for cultures EMB₂ 166, 168 and 173 in particular, Table I. For the fermentation of dextrose and mannose by these three strains there appears to be a specific value in the peptic digests of the B.D.H. caseins—a value that is not apparent in the digest of Will Corporation commercial casein; source No. 1, Table I. As was shown for the peptic casein digest broth prepared from Will Corporation commercial casein, Table I, so with the peptic casein digest broth prepared from acetic precipitated soluble casein (B.D.H.) and light white soluble casein (B.D.H.) respectively—the broth containing the greater total nitrogen content (1) being the more suitable source of nitrogen for the fermentation studies on the five cultures, Table I. Both the "kind" and "amount" of nitrogen supplied are seen to be important.

On digesting Vitamin-Free casein (B.D.H.) with pepsin, it has been seen that the yield of available nitrogen is little more than half that obtained by the peptic digesting of light white soluble casein (B.D.H.) (1). We find however that the nitrogen distribution of the two is somewhat of the same order (1); and that the peptic digest broth prepared from Vitamin-Free casein (B.D.H.), source No. 12, is comparable in value as a nitrogen source for the five cultures, with the diluted peptic digest broth prepared from light white soluble casein (B.D.H.), source No. 17, Table I, (see also (1) Table I). These comparisons are of peculiar interest.

The peptic casein digest broth, source No. 18, prepared from casein (Plimmer) provides a comparatively small amount of the subpeptone nitrogen fraction (1). The digestion continued for 10 days (1); yet it has been seen that

the nitrogen distribution is almost identical with that of source No. 8, a peptic casein digest broth prepared from commercial casein (Will Corporation) by digesting for two days only (1). When employing the diluted broth of each, Nos. 19 and 9, as nitrogen sources for the fermentation studies, the total titratable acidity produced by cultures EMB₂ 166, 168 and 173 respectively is low and almost identical in amount, Table I: low total titratable acidity when supplied with a nitrogen source poor in the subpeptone nitrogen fraction. As a nitrogen source for cultures EMB₁ 173 and 195, source No. 19 is comparable in value with source No. 2, the diluted peptic digest broth prepared from commercial casein (Will Corporation), Table I.

If now we compare No. 19 with No. 2—the diluted peptic digest of commercial casein (Will Corporation)—each broth being the result of a 10-day period of digestion (1), the former is seen to provide little more than half the amount of subpeptone nitrogen yielded by the latter; the differences being small in the peptone nitrogen and proteose nitrogen fractions respectively (1). The differences in the nitrogen distribution are reflected in the influence on the sugar-fermenting abilities of three of the organisms. Supplied with No. 19 as the nitrogen source, cultures EMB₂ 166, 168 and 173 produce less total titratable acidity than when supplied with No. 2, Table I: the lower amount of acid being produced when the nitrogen source containing the lesser subpeptone nitrogen fraction is supplied. No such significance is apparent in the case of cultures EMB₁ 173 and 195, Table I.

Although the peptic digest of blood fibrin, source No. 24, contains less proteose nitrogen and much more peptone nitrogen than, say, the peptic casein digest source No. 1 (1), the former is much less suitable as a nitrogen source for cultures EMB₂ 166 and 168 than is the latter, Table I—the provision of an adequate supply of the subpeptone nitrogen fraction and the peptone nitrogen fraction notwithstanding. On the other hand, blood fibrin digest is slightly superior to the casein digest as a nitrogen source for cultures EMB₁ 173 and 195 and culture EMB₂ 173, Table I. Of necessity, the diluted broth of each source has been considered in these comparisons. The subpeptone nitrogen and peptone nitrogen fractions in the peptic blood fibrin digest apparently fail to influence the sugar-fermenting abilities of cultures EMB₂ 166 and 168 to the same extent as do these fractions when contained in a peptic casein digest, Table I (see also (1) Table I). Hence it may be that suitability as a nitrogen source for fermentation studies is not fully indicated by the nitrogen distribution picture of the source.

It has been seen that the tryptic casein digest broth, source No. 20, is made up of 2.50% protein nitrogen, 27.68% peptone nitrogen and 69.82% subpeptone nitrogen; and that there is an entire absence of the proteose nitrogen fraction (1). The broth containing approximately 1% nitrogen is unsuitable as a nitrogen source for any of the five cultures, Table I. The diluted broth, total nitrogen content approximately 0.5%, source No. 21, is also quite unsuitable as a nitrogen source for cultures EMB₁ 173 and 195, Table I. On the other hand, when the dilute broth is employed, the total titratable acidity produced by cultures EMB₂ 166, 168 and 173 is high, Table I—interestingly

enough, the figures being comparable with those obtained when the nitrogen source is the peptic digest broth (0.908% total nitrogen) prepared from light white soluble casein (B.D.H.), Table I: and the difference in the nitrogen distribution in the two sources is marked. Again there are clear indications that both the "kind" and "amount" of nitrogen supplied are critical. In the two concentrations, the kind of nitrogen available in the tryptic casein digest is unsuitable for cultures EMB₁ 173 and 195. In the higher concentration, tryptic casein digest broth is unsuitable for cultures EMB₂ 166, 168 and 173; yet when the diluted broth is used, not only the "kind" but also the "amount" of nitrogen contained is particularly suitable as a nitrogen source for the fermentation of the sugars by these cultures, Table I.

Witte's peptone broth, source No. 26, is low in the subpeptone nitrogen fraction and contains a higher percentage of protein nitrogen than does any other source analyzed (1). Provided the total nitrogen content of the broth is 1%, Witte's peptone is suitable for cultures EMB₁ 173 and 195, but unsuitable for cultures EMB₂ 166, 168 and 173; diluted to contain 0.5% total nitrogen, source No. 27, Witte's peptone broth is inadequate as a nitrogen source for any of the five cultures, Table I.

It may be seen (1) that the nitrogen distribution in proteose peptone (Difco) broth, source No. 28, and Bacto peptone broth, source No. 32, is somewhat of the same order, and is dissimilar from the distribution found in any other source analyzed. Also it is seen (1) that both sources contain much more peptone nitrogen, more subpeptone nitrogen and much less proteose nitrogen than the peptic digests of casein contain (1). Except for the fermentation of mannose by cultures EMB₂ 166 and 168, Bacto peptone broth—or the same broth diluted, source 33—is inadequate as a source of nitrogen for determining the sugar-fermenting abilities of any of the five cultures, Table I. On the other hand, proteose peptone (Difco) broth, 1% total nitrogen content, is a good nitrogen source for each of the five cultures and, nitrogen content for nitrogen content, compares favorably with the peptic casein digests, Table I. In common with the peptic casein digest prepared from light white soluble casein (B.D.H.), source No. 16, proteose peptone (Difco) broth appears to have a specific value as a nitrogen source for the fermentation of mannose by cultures EMB₂ 166, 168 and 173, Table I (see also (1)).

From Table I, and (1) it is seen that, showing a nitrogen distribution picture somewhat of the same order, proteose peptone (Difco) broth and Bacto peptone broth are not at all comparable, the one with the other, when the value of each as a nitrogen source is reflected in the sugar-fermenting abilities of the five cultures, Table I. Again there is the suggestion that the nitrogen distribution in a source does not necessarily give a full indication as to the suitability or otherwise of the source for the determining of the sugar-fermenting abilities of these cultures (1), Table I.

In Bacto yeast extract broth, source No. 34, the nitrogen content consists entirely of subpeptone nitrogen 75.80% and peptone nitrogen 24.20%, the subpeptone nitrogen fraction being a little greater and the peptone nitrogen fraction a little less than these fractions respectively in tryptic casein digest,

source No. 20 (1). As has been seen (1), apart from a small percentage of protein nitrogen in tryptic casein digest, the two sources are almost identical in their nitrogen distribution. This marked similarity is reflected in the influence on the sugar-fermenting abilities of cultures EMB₁ 173 and 195. The yeast extract broth, as the tryptic casein digest broth, is unsuitable as a nitrogen source for the fermentation of the three sugars by these two cultures—whether the original broth (1% total nitrogen content) or the diluted broth (0.5% total nitrogen content) source No. 35, be employed, Table I. For cultures EMB₂ 168 and 173, yeast extract broth having a total nitrogen content of 1% is much more suitable as a nitrogen source than is tryptic casein digest broth containing approximately 1% nitrogen, but much less suitable when the diluted broth is used, Table I. Yeast extract broth does not compare favorably with peptic casein digest broth or with hydrolyzed casein (Difco) broth as a nitrogen source for the fermentation of glucose and lactose by cultures EMB₂ 166, 168 and 173, but there is a specificity in yeast extract as a nitrogen source for the fermentation of mannose by these three strains, Table I (see also (1) Table I)—a specificity comparable with that observed when discussing Bacto peptone broth and the peptic digests of casein (B.D.H.).

The unsuitability of marmite as a nitrogen source for each of the five cultures is a curious finding, Table I, particularly when the nitrogen distribution picture is compared with the nitrogen distribution in yeast extract broth and in tryptic casein digest broth respectively (1). The authors can offer no comment.

In its nitrogen distribution, hydrolyzed casein (Difco) broth presents something of a composite picture of the nitrogen distribution in a peptic casein digest and a tryptic casein digest (1), and when employed as a nitrogen source reflects this composite quality in the influence on the total titratable acidity produced from each of the sugars by each of the five cultures, Table I. Hydrolyzed casein broth is found to contain less proteose nitrogen, more peptone nitrogen and much more subpeptone nitrogen than the peptic casein digests Nos. 1, 14 or 16 contain; and, as compared with the tryptic casein digest, less than half the amount of subpeptone nitrogen and 35% more proteose nitrogen (1). Employed as a nitrogen source for cultures EMB₁ 173 and 195, hydrolyzed casein broth is comparable with the peptic casein digests, sources No. 1, 14 or 16, Table I. For cultures EMB₂ 166, 168 and 173, hydrolyzed casein broth is the most suitable source of nitrogen we have investigated, Table I. The influence as a nitrogen source is greater when the broth containing 1% total nitrogen rather than 0.5% total nitrogen is used, Table I. The pronounced suitability of hydrolyzed casein broth as a nitrogen source for the fermentation of the three sugars by cultures EMB₂ 166, 168 and 173 is made quite clear by the data in Table I: the diluted broth, source No. 31, is approximately as suitable a nitrogen source as are the peptic digests of casein containing 1% total nitrogen; and as suitable as the tryptic casein digest broth containing approximately 0.5% total nitrogen, Table I. Again it is made clear that both the "kind" and the "amount" of nitrogen supplied are important.

Of itself, hydrolyzed casein broth, employed as a nitrogen source for the fermentation of the three sugars used, differentiates as between cultures EMB₁ 173 and 195 on the one hand and cultures EMB₂ 166, 168 and 173 on the other hand, Table I.

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NOTE ON AN INVESTIGATION INTO THE FLUORESCENCE OF HAIRS INFECTED BY CERTAIN FUNGI¹

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Abstract

1. Observations under filtered ultra-violet light of the available material of *Microsporon audouini*, *M. felineum*, *Trichophyton gypseum*, *T. violaceum*, *T. album* and *Achorion schoenleini* indicate that the species belonging to the genera *Microsporon* and *Achorion* produce an intense green fluorescence in the substance of hairs infected by them. *Trichophyton*-infected hairs appear a paler bluish-white. Normal pigmented hairs appear dark.

2. It is shown that the spores and hyphae of the fungus whether within or without the hair are relatively non-fluorescent as contrasted with the substance of the infected hair.

3. A fluorescent substance present in hairs infected by *M. audouini*, *M. felineum* and *Achorion schoenleini* is readily soluble in hot water. No such substance could be extracted from the hairs infected by *Trichophyton sp.*, or from normal hairs. It is therefore believed that the presence or absence of the green fluorescence is pathognomonic.

Introduction

In 1925 Margarot and Devèze (3) observed the appearance of a green luminosity of the hairs from cases of favus and microsporiasis examined in a darkened room by means of the ultra-violet radiation passing a filter of Wood's glass. The phenomenon was subsequently investigated by Vigne (6), Kinnear (2) and others, and the relevant literature up to 1929 is discussed in a later paper by Margarot and Devèze (4).

That the phenomenon has been utilized in the diagnosis of cases of ringworm of the scalp is shown by the numerous clinical references which have appeared during the last few years. The source of light usually employed is a mercury-arc, or carbon-arc lamp, fitted with Wood's glass or some other filter which has the property of absorbing most of the visible light and transmitting the long-wave ultra-violet radiation lying near to the visible region of the spectrum. A convenient portable lamp for this purpose has been developed by the present writers and described in another communication (1). If the beam of filtered ultra-violet light is allowed to fall upon the head of a child suffering from tinea capitis caused by *Microsporon audouini*, the affected areas are observed to shine with an intense emerald-green color, contrasting strongly with the normal parts of the scalp where the hairs usually appear dark or reflect some of the dim violet light which is not completely absorbed by the filter.

The present investigation is being carried out in order to determine whether or not fluorescence affords an accurate and rapid aid to diagnosis and a precise criterion of cure, as a preliminary step in a study of methods of

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treatment of ringworm of the scalp. Particular attention is being paid therefore to the limitations of the phenomenon in diagnosis, as a knowledge of these is necessary if valid conclusions are to be drawn from the test. The problem is being investigated in order to discover which species of fungi do, and which do not, cause the infected hairs to fluoresce, to determine how far infection is co-extensive with fluorescence, and to throw some light on the nature of the phenomenon.

The Fluorescence

Fluorescent hairs were studied in the laboratory by means of a microscope illuminated with a Leitz "Luminescence Lamp". This instrument consists of a carbon-arc lamp fitted with suitable filters which transmit ultra-violet light, but absorb visible radiations except those at the extreme violet and red ends of the spectrum. Another filter fitting over the eyepiece of the microscope is a complementary absorbent to the lamp filters, except in the extreme red region of the spectrum. Thus, on looking down the microscope tube, the empty field of view appears dark except for a red tinge. As the lamp and eyepiece filters are complementary absorbents, luminosity of an object placed in the field of the microscope must be due to fluorescence. As has been noted by previous writers, normal hairs examined by filtered ultra-violet light show a bluish-white fluorescence of various intensities, or appear dark, according to the absence or presence of pigment in the hair. A similar bluish-white fluorescence is noted in hairs infected by *Trichophyton*. Hairs infected by *Microsporon audouini*, or *M. felineum* fluoresce with a brilliant green color. By means of a Hilger Constant Deviation Spectroscope, preliminary examinations have been made of the fluorescent light emitted by uninfected white hairs, and by hairs attacked by *Achorion schoenleini*. Observations were difficult because individual readings differed considerably according to the degree to which the eye of the observer was accommodated to the very faint spectrum. A further inaccuracy was caused by the obvious differences in intensities of the fluorescent emissions from normal and infected hairs. The radiation from both sources consisted of a faint wide band extending from the yellow into the violet. At the yellow end, both bands terminated fairly sharply in the same region. The band spectrum of the uninfected hair, however, passed slightly farther into the violet than did that of the diseased hair. It remains to be shown by further studies whether this difference is enough to account for the difference in color between the hairs as perceived by the eye, or whether the colors may be in part an example of Purkinje's phenomenon caused by differences in the intensities of the emitted light. Evidence will be brought forward later to show that there is a difference in nature, if not necessarily in hue, between the fluorescence of normal, of *Microsporon*-infected, and of *Trichophyton*-infected hairs.

The phenomenon appears to fulfil two of the conditions which characterize fluorescence. (a) The light emitted by the luminescent hairs (green), to judge by the apparent hue, is of longer wave-length than the light exciting the emission (violet or ultra-violet). This conclusion is borne out by the fact that the

luminescence is not extinguished by the use of complementary absorbents. In the absence of direct spectroscopic measurements, however, the term "fluorescence" is applied to the phenomenon rather for convenience than as a result of strict proof. (b) The emission of the light appears to cease directly the exciting radiation is removed.

Relation between Fluorescence and Species of Fungus

Isolations of fungi have been made from hairs obtained from cases of ringworm of the scalp, beard, and trunk, and the organisms determined in culture upon Sabouraud's maltose "Proof" medium. The results of this study as far as they go are quite definite, and show that the species of fungus involved is the chief factor determining the presence or absence of fluorescence in the infected hair.

TABLE I
CORRELATION BETWEEN SPECIES OF FUNGI ISOLATED FROM
INFECTED HAIRS AND EMERALD-GREEN FLUORESCENCE
UNDER ULTRA-VIOLET LIGHT

Organism	No. of cases from which isolated	No. of cases showing green fluorescence
<i>Microsporon audouini</i>	38	37*
<i>Microsporon felineum</i>	17	17
<i>Trichophyton album</i>	3	0
<i>Trichophyton gypsum</i>	2	0
<i>Trichophyton violaceum</i>	4	0
<i>Achorion schoenleini</i>	4	4

*+1 Doubtful.

microscopical grounds, but were not grown and determined in culture. Table II shows the correlation between genus of parasite and the production of the green fluorescence in these cases where the study has been less thorough.

As indicated above, hairs invaded by *Trichophyton* species frequently show a bluish-white fluorescence of lower intensity than the green fluorescence of hairs infected by *Microsporon*.

These observations on the occurrence of the fluorescence show that: (a) the presence or absence of the green fluorescence in infected hairs is usually determined by the species of fungus infecting the hair; (b) infection by *Microsporon audouini*, *M. felineum*, or *Achorion schoenleini*

Table I shows the correlation between the species of fungi isolated from ringworm-infected hairs, and the presence or absence of the emerald-green fluorescence in ultra-violet light.

In addition to the above cases from which the fungi have been isolated and identified in culture, there are a number of cases in which the organisms were classified as *Trichophyta* or *Microspora* on clinical and

TABLE II
CORRELATION BETWEEN GENUS OF PARASITIC FUNGUS AND
PRODUCTION OF FLUORESCENCE

Organism	No. of cases observed	No. of cases showing green fluorescence
<i>Microsporon</i> species	23	23
<i>Trichophyton</i> species	10	0

results in a strong green fluorescence; (c) infection by the species of *Trichophyton* studied causes a fluorescence resembling that of the normal stratum corneum.

Other Factors Conditioning Fluorescence

Although Tables I and II indicate that the species of parasite is largely responsible for the presence or absence of the green fluorescence, one doubtful case is recorded. This patient suffered from ringworm of the scalp caused by *Microsporon audouini* and was in contact with a number of other children similarly infected, all of whom showed the typical bright green fluorescence associated with *M. audouini*. When first examined, no trace of fluorescence could be observed over the areas in which typical broken hair-stumps could be found by means of a hand-lens. About 14 days later, the infected hairs showed a feeble green fluorescence when examined by the light of a water-cooled mercury-arc lamp fitted with a Wood's filter. Two weeks later the fluorescence had entirely disappeared. No satisfactory explanation for this variability in fluorescence is known to us.

In cases of infection by *Microsporon audouini* which were kept under observation over several months, variations were noted from time to time in the relative intensity of the green fluorescence manifested by different patients.

These observations are the only facts yet encountered which suggest that differences in the individual patient may sometimes determine the presence or absence of the fluorescence.

The Location of the Fluorescent Substance

An explanation of the fluorescence has been given by Margarot and Devèze (4). "La fluorescence propre des divers agents des teignes explique la teinte verdâtre observée en pareil cas. Les recherches de Vigne et les nôtres établissent que la luminosité est due au champignon lui-même. Nous avons pu nous rendre compte que dans le monde végétal d'autres champignons microscopiques étaient fluorescents." From this conclusion follows the view that the apparent non-fluorescence of the endothrix *Trichophyta* is due to the suppression of the luminosity by the matter surrounding the fungal hyphae distributed within the hair. Thus Vigne (6) wrote, "La fluorescence des cheveux teigneux étant due à la présence de spores, il n'est pas étonnant de constater que les cheveux contaminés par le Trichophyton, parasite endothrix, sont moins lumineux que ceux contaminés par le Microsporon dont les spores sont en très grand nombre et situées tout autour du cheveu." These explanations are regarded by the present writers as inadequate for the following reasons.

(1) In *Trichophyton* the ectothrix species *T. gypsum* and *T. album*, as well as the endothrix *T. violaceum*, do not exhibit the green fluorescence.

(2) Hairs infected by *Achorion schoenleini* in which the hyphae are situated almost entirely within the substance of the hair show the green fluorescence in a marked degree.

(3) The green fluorescence is not observed macroscopically in cultures of the organisms when grown on Sabouraud's medium. The cultures frequently

appear a violet color under the Wood's light, and a beautiful golden brown fluorescence has been noted from a culture of *Microsporon felineum*.

Moreover, for the following reasons the luminosity is believed to lie primarily within the substance of the hair.

(1) Microtome sections of hairs attacked by *Microsporon audouini* have been cut in the region of the follicle and spore sheath. By ultra-violet light with complementary filters, the whole section was visible, the follicle and spore-sheath appearing faintly bluish-white and the hair shaft bright green.

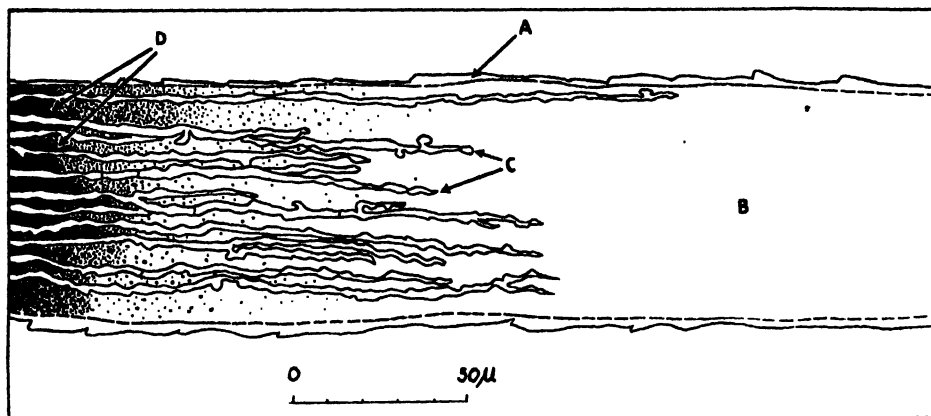


FIG. 1. Semi-diagrammatic camera lucida drawing of region of Adamson's fringe in a hair infected by *Achorion schoenleini*, showing the relation between the hyphae of the fringe and the blue-green fluorescence (represented by shading) excited in the infected portion of the hair by the rays passing a filter of Wood's glass. A, cuticle of hair; B, uninfected portion of hair shaft between the bulb of the hair and infected region; C, the tips of the growing hyphae invisible by the Wood's light; D, the fluorescent part of the hair represented in reverse by shading, first appearing some little distance behind the edge of the fringe.

(2) Microtome sections have been cut through the distal ends of hairs infected by *Achorion schoenleini*. Here the hyphae have mostly disintegrated, leaving channels in the keratin filled with air or with fat droplets. When examined microscopically with filtered ultra-violet light the cuticle and substance of the hair were seen to be fluorescent.

(3) Hairs infected by *Achorion schoenleini* were epilated intact and examined microscopically by filtered ultra-violet light in the region known as "Adamson's Fringe", where the invading fungus terminates as a fringe of hyphae just above the bulb of the hair. The fringe is thus the region of active invasion of the hair. The hyphae forming the fringe were invisible by ultra-violet light. The luminous portion of the hair "faded in" gradually at a short distance behind the fringe which is the region of active invasion. As illustrated semi-diagrammatically in Fig. 1, the hyphae are non-luminescent but the hair becomes luminous usually some little time after it is actually penetrated by the hyphae.

(4) Evidence was also obtained from a study of hairs newly infected by *Microsporon felineum*. It has been shown by Sabouraud (5) that before a hair is actually penetrated by the hyphae of *Microsporon*, broad ribbon-like hyphae

descend into the horny layers of the follicle. Some distance below the mouth of the follicle, the hyphae send branches inwards which enter the hair. Subsequently the mycelium grows downwards in the shaft of the hair, branching in the direction of the root. The part of the hair-shaft penetrated by the hyphae is covered by a sheath of spores developed from the infra-cuticular mycelium (Fig. 2, A). Such newly infected hairs were epilated together with the greater part of the follicle but not with the root. These were examined microscopically with filtered ultra-violet light. It could then be seen that only that part of the hair actually penetrated by the fungal hyphae was fluorescent (Fig. 3). The external mycelium surrounding the hair-shaft in the upper part of the follicle did not itself fluoresce or induce fluorescence in the hair substance beneath it (Fig. 2, B). The spore sheath (Fig. 2, A) appeared but feebly fluorescent (Fig. 3, A). The lower part of the hair where the spore-sheath had fallen off in epilating the hair showed that the hair-fibres were intensely fluorescent (Fig. 2, C and Fig. 3, C).

(5) The hyphae running longitudinally through hairs infected by *Achorion schoenleini* and *M. audouini* when examined microscopically by filtered ultra-violet light usually appear duller, but never, so far as observed, brighter than the adjacent part of the hair.

The conclusion drawn from the observations cited above is that the fluorescence is due to some change in the hair substance following invasion by the fungus. It is possible that an enzyme is excreted by the hyphae into the surrounding medium, and that some product of hydrolysis of the keratin or other body present in the hair may be the fluorescent substance.

The Fluorescent Substance

From *Achorion schoenleini*, *Microsporon audouini* and *M. felineum* a fluorescent substance has been extracted and obtained in the form of a fluorescent aqueous solution. Repeated attempts to extract a similar substance responsible for the pale blue or white fluorescence exhibited by hairs infected by *Trichophyton* species, or by normal hair, have been unsuccessful.

An experiment which has been repeated many times may be cited. From a scalp infected with *Achorion schoenleini* a small quantity of hair, about 0.2 mgm., was clipped from an infected area. A like sample was taken from an adjacent area of normal hair on the same scalp. When examined by means of the filtered ultra-violet light, the infected hairs were found to fluoresce while the normal hairs appeared dark. Any fluorescent hairs that had been gathered with the normal control sample could be removed at this stage. The samples were covered with ether and allowed to stand overnight. This treatment removed the natural fat of the hair. From the infected sample the fatty substance filling the channels in the hair, no doubt a product of fungal metabolism, was removed also. The removal of the fat allowed of wetting the hair thoroughly in the subsequent extraction. The ether was poured off, and the ethereal extracts from both the normal and the infected hairs could be seen to be non-fluorescent though they appeared faintly opalescent and

reflected some of the light which passed the filters. The appearance of the hairs by filtered ultra-violet light remained unchanged.*

The ether extracts containing the fat were evaporated down and clustered stellate fat-crystals soon appeared. The fat from both samples failed to show the green fluorescence. The dried samples of hair were then placed in test tubes, covered with about 10 cc. of distilled water, and heated in a water bath for a period of about an hour; the liquid was then filtered off and a further portion of water added to complete the extraction. The tubes were examined from time to time by the ultra-violet light, to check the progress of the extraction. When the filtered extracts from the two samples were thus examined, that from the infected hair was seen to fluoresce with a bright green color, while that from the normal hair was colorless. The nature of the fluorescent substance is under investigation.†

TABLE III

COMPARISON OF MICROSCOPICAL APPEARANCE OF HAIRS INFECTED BY *M. audouini* AND *T. gypseum*, WHEN SUBJECTED TO VARIOUS TREATMENTS SUCCESSIVELY

Examined dry with luminescence lamp	Water run under cover-slip	Boiled 1—1.5 min.	Dried in ether, examined again
<i>Microsporion audouini</i>			
Intense green fluorescence of hair. Spore sheath apparently transparent	No change	Fluorescent color diffused out into the water, specimen left very dull-looking	No change
<i>Trichophyton gypseum</i>			
Bluish-white fluorescence pronounced in corneous layers of follicle. Hair dark except at infected end which was somewhat blue-fluorescent, but less so than follicle.	Fluorescence of follicle dimmed at once. Hair became transparent, still appeared dark, except at infected end where feeble fluorescence was unchanged.	No change	Bluish-white fluorescence of follicle and infected portion of hair returned

* Incidentally when the hairs were allowed to dry after removing the ether, the infected hairs stood out prominently by reason of their lustreless grey color. This probably explains the well-known clinical test in which the scalp is washed with chloroform and the infected hairs are then seen to be frosted-white in appearance. The chloroform no doubt extracts the fatty substance present in the empty tubes left in the hair substance after the death of the fungus. When the fat solvent evaporates, the tubes become filled with columns of air. The greatly increased surface of hair-substance in contact with air reflects more light.

† A preliminary chemical examination of the fluorescent extract has been made by Peter G. Mar, M.Sc. The aqueous extract obtained by the above procedure was concentrated by distillation at 35°—40° C. under reduced pressure. The concentrate (ca. 1 cc.), a light yellowish clear solution, still fluorescent under the filtered ultra-violet radiation, was then placed in a vacuum desiccator and dried over concentrated sulphuric acid. A small quantity of a somewhat hygroscopic substance remained, white, amorphous, powdery, and having a sharp musty odor.

Preliminary chemical tests with a small amount of the fluorescent solution indicated the presence of a neutral organic substance, or substances, containing nitrogen, an aldehyde group and a phenolic ring. Tests for carbohydrate, loosely combined sulphur and α -amino acids were negative. However, these results are put forward only provisionally, as the quantities used were too small to allow of purification and identification.

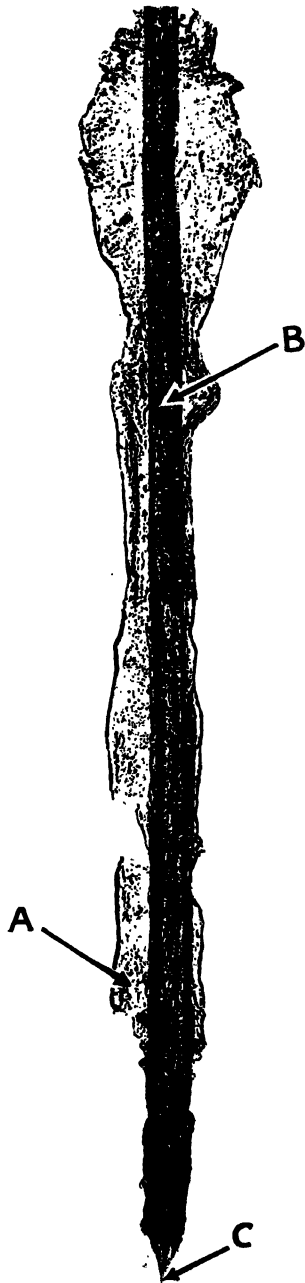


FIG. 2. Hair and follicle without hair-bulb in early stage of infection by *Microsporon felinum*, photographed by visible light. A, the spore sheath surrounding the part of the hair penetrated by the fungus; B, the uninvaded portion of the hair shaft, outside which, however, the fungal hyphae could be seen on staining. C, the broken end of the hair.

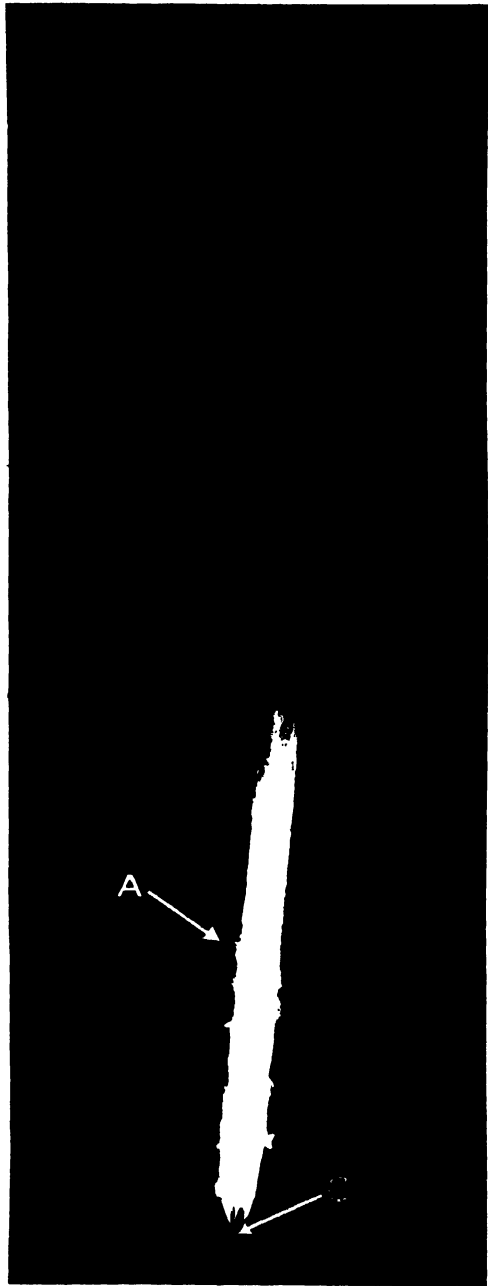


FIG. 3. The same specimen photographed by ultra-violet light. A, faint fluorescence of the spore sheath; C, the broken end of the hair showing fluorescent hair fibres. The intensity of the fluorescence of the infected part of the hair fades rapidly towards the uninfected portion.

Contrary to the experience of Kinnear (2), who found that the infected hairs retain their fluorescence in liquor potassae, it has been found that the extraction of the fluorescent substance may readily be demonstrated microscopically for single hairs by mounting them dry on a slide and running a drop of 7% potassium hydroxide under the cover-slip. The hairs are examined by ultra-violet light. Almost at once the fluorescence in the hair begins to fade, while the liquid in which the hair is mounted takes up the fluorescence.

Repeated comparisons have been made of the microscopical appearance of green-fluorescent hairs infected by *M. audouini* and the dull, bluish-white fluorescence of hairs infected by *T. gypseum*, when treated with boiling water. Typical observations are shown in Table III.

From these experiments it is concluded that a fluorescent substance can be extracted by warm water from hairs infected by *Achorion schoenleini*, by *Microsporon felineum* and by *Microsporon audouini*, but not from hairs infected by *Trichophyton gypseum*. The bluish-white fluorescence of these *Trichophyton*-infected hairs cannot therefore be due to the presence in smaller amount of the same substance as that producing the intense green fluorescence in hairs infected by *Achorion* and *Microsporon*. The difference observed between hairs infected by *Microsporon* sp. or *Achorion* sp. and hairs infected by *Trichophyton* sp. when examined by Wood's light is therefore considered to be a difference in kind and not merely of degree.

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IS THE DOOR OF *UTRICULARIA* AN IRRITABLE MECHANISM?¹

BY FRANCIS E. LLOYD²

Abstract

The present paper deals with the question whether the door mechanism in *Utricularia* is an irritable one (as *e.g.*, *Mimosa* is irritable) or is mechanical, and in the opening paragraphs the two views are set forth together with mention of the protagonists and their factual contributions. In particular the recently published views of Kruck have been examined and found to be inadequate.

(a) The evidence for the conclusions herein presented have been drawn from a study of the posture of the door, and its exact positional relations to the threshold. The correlated anatomical facts have been studied and set forth, and it has been shown that the physical properties of the door do not change during action and that its action depends wholly on the pressure of the external medium (water) induced by the out-pumping capacity of the walls of the trap, first observed by Brocher, on the water-tightness of the door procured by the velum, as shown by Lloyd, and upon the actuation of a tripping mechanism also earlier described by the same author. There are no changes in turgor in the door cells and no reception or transmission of stimuli by the bristles, which are simply a part of the tripping mechanism.

(b) Evidence has also been obtained from a study of the flexures and movements of the door, and it is shown that such could not occur if there were changes in turgor. It is further shown that the histology of the door and threshold are correlated with the position, degree and direction of the flexures.

(c) It has been shown that during the flexures and movements of the door, there is no change in the position or form of the air contained in the intercellular spaces of the door, such as have been said to occur as a result of the movement of sap into these intercellular spaces.

(d) Killing of the bristles does not prevent the action of the door, although it has been postulated that the bristles receive and transmit stimuli to the tissues of the door.

(e) The rate of movement of the door during action is so rapid that, while it does not preclude the interpretation that it is an irritable response, it at least exposes it to grave doubt. Since the action can be repeated as rapidly and as often as the experimental conditions permit there can be no period of recovery. The action is so rapid that, if the mechanism is an irritable one, the latent period, or the period of transmission, must be very much shorter than any other case in the plant kingdom.

The conclusion is therefore reached that the mechanism of the door is purely mechanical, though this is not to say that the trap is purely passive, since the physiological activity of the walls has to play its part in exhausting water from the interior, as a reduced water pressure within is necessary for the action of the door.

A comparative study of 75 species of *Utricularia* supports this conclusion.

Foreword

In a paper already published in 1929, I gave a rather full account of the door mechanism of *Utricularia gibba*. This description was found to apply, except in regard to non-essential details, to most of the freely floating species, of which *U. vulgaris* is perhaps the most widely distributed and best known to botanists in general. In following papers I therefore did not use space for adding an extended description of *U. vulgaris* and its like. But most of the work of Continental students has been done on this species, so I have bethought

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myself that it would serve a useful purpose to give a fuller account of it. The recent publication of a view about the nature of the door mechanism quite divergent from my own, set forth in my 1929 publication, has supplied the occasion of combining an answer to that view with a description of some exactness, I venture to hope, of the anatomy, histology and functioning of the door mechanism, particularly of *U. vulgaris* and *U. intermedia*.

Such descriptions are difficult of achievement if we are confined to words alone, which, however adroitly used, put a heavy onus of constructive visualization on the reader. Diagrams are of much help, but a look through the microscope would be greatly better. The next best are good photographs, which, without any illusions about their necessary verity, can be used to make visualization relatively easy. To this end the present account is supported by a rather abundant supply of photographs each of which was made to clarify to the reader (as they have originally to the present writer) the point of description correspondingly set forth.

Introduction

There have naturally been two views of the nature of the action of the door of *Utricularia*; that, on the one hand, it is an expression of irritability, the bristles appearing to correspond to the sensitive hairs of *Aldrovanda* and of *Dionaea*; on the other, that the whole behavior is purely mechanical. The latter view has been supported latterly by Merl (23) (though with some caution), by Czaja (5) and by myself (18, 19); Merl, followed by Czaja, especially having investigated the mechanism by means of experiments designed to differentiate between the two types of mechanism. Although the issue seemed to be closed, I repeated to my own satisfaction many of their observations, and then proceeded to study the structure of the trap in the light of the physiological facts apparently demonstrated as true. I showed that previous notions as to the positional relations of the door to the threshold were incorrect, and further, that the water-tightness of the door is procured not by mucilage, but by the presence of a membrane, the velum, derived by partial exfoliation of the collective cuticle of the pavement epithelium, to use Goebel's term, in such manner that the resulting membrane remains attached to the forward three to five courses of cells of this epithelium (Plates III, IV, V). Later, I extended my examination to about 75 species of *Utricularia* and to *Polypompholyx*, from the results of which it became evident that the original account which I gave for *U. gibba* (Lloyd, 18) furnished a clue which enabled me to explain the minutiae of structure of these numerous forms. Taking all the varieties of structure into consideration, the impression was continually emphasized that the conclusion first experimentally supported by Merl was correct.

The contrary view, that the mechanism is an irritable one, in particular that the bristles, the touching of which discharges the trap, are sensory hairs and that the door and even much adjacent tissue are "motor" in nature, has been advocated by Brocher, by Ekambaram and by Withycombe (26). The two latter, curiously enough, supplied evidence which is antagonistic to their own notions, to be mentioned in detail beyond. It cannot be said that any

convincing evidence was advanced by any of these; rather it was a natural interpretation prompted by general knowledge. More recently, M. Kruck (17) has come forward in support of the same view, and advances evidence to support the contention that the bristles are in fact sensitive hairs, that these and the cells of the door are irritable and that the initial action of the bristle-door system consists in the reception, followed by the transmission of the stimulus by and through the bristles and through their bases to the cells of the door. It is claimed that in consequence the cells of the door contract, forcing water into the intercellular spaces, thus procuring the shrinkage of the tissues which effects a release of the door edge from the threshold, when the pressure of water completes the action. Kruck thus attempts to combine the indubitable fact that a reduced pressure of water, furnishing an unstable equilibrium, exists in the trap, as first observed by Brocher (1), with the view that the release of the door from its tight application to the threshold is an irritable response. This author further gives an account of the structure of the door and of its posture with relation to the threshold, accompanied by diagrams (Fig. 14) to make her meaning clear. Although it is quite thinkable that the door is an irritable mechanism, whatever be its posture, so that these two considerations must be regarded separately, nevertheless, Kruck has joined these two issues as part and parcel of her general contention. In what follows I shall consider first, the door; (a) its posture, (b) its structure; and secondly, the question whether it is purely physical-mechanical in its action, or is a sensitive-irritable mechanism.

Material

Kruck does not state the species studied by her, but from her sketches it was evidently *U. vulgaris* or a nearly related form. I have studied living material of *U. aff. gibba*, of *U. intermedia* and of *U. vulgaris* to the present purpose. All of these are in essential features, to all intents and purposes, identical.

Approach to the Problem

A general description of the trap or "bladder" should not be necessary here. A good one of *U. vulgaris*, though, in the light of more recent studies, faulty in

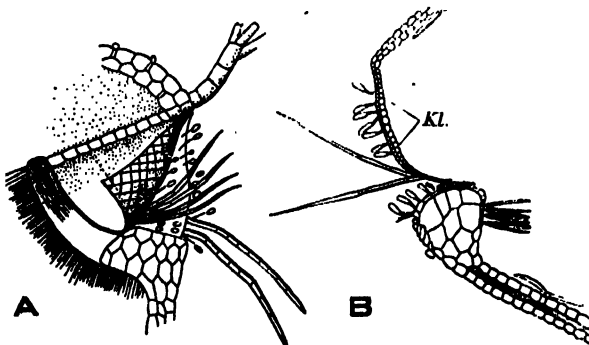


FIG. 1. (A) "Median section" through trap of *U. vulgaris*. After Cohn (3). (B) *U. flexuosa*. After Goebel (15).

certain respects, has been given by Skutch (25) in an article summarizing our knowledge to the date of his writing. Others are found in the papers of Merl, Czaja and Kruck. It need only be recalled that the trap of this type is a compressed hollow pear-shaped affair, with the door and threshold at the, as it were,

truncated, narrow end, and supported on a stalk attached on one (ventral) side. The margin of the entrance is furnished with putative guides for prey, two antennae and lateral bristles, which need not be considered here. When the trap is in the "set" condition, the sides are concave (Brocher), as seen in Plate VI-55, and Fig. 4, due to the fact that the walls have the power of pumping out the water from the interior and thus lowering the water pressure within*. The greater external water pressure is exerted equally on the door, of course, but this is so constituted and postured that it can resist the pressure. The door is indeed water-tight (Brocher, Merl, Czaja). This property has been attributed to the firmness with which the door presses on the threshold (Brocher, Fig. 4, Czaja, Fig. 5) and to the presence of mucilage (Brocher). Before this water-tightness was known to be a fact, the door, since the time of Cohn and C. Darwin was thought to be a simple valve, under the edge of which minute animal forms could creep to their ultimate fate, often destruction. This seems indeed to be true of *Polypompholyx* (Lloyd, 20) but is not true of any of the 75 species of *Utricularia* studied by me so far. It is conceded, then, that the door is in a firm position of some sort, and requires some initial alteration so that the condition of unstable equilibrium (as Brocher first called it) may be disturbed. This initial alteration is procured in the species we are considering by some pressure on the bristles which, four to six in number, are inserted near the middle line of the door and near its lower free edge (Figs. 7, 13). Does such pressure constitute a "stimulus" which is transmitted by the protoplasm down the bristles, through their bases, and so to the cells of the door? Or does it merely cause a physical alteration of the shape of the door, so upsetting the unstable equilibrium?

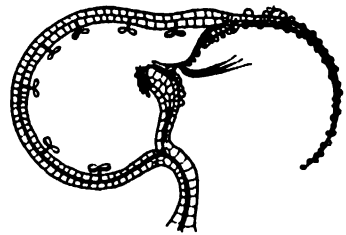


FIG. 2. *U. reniformis*.
After Luetzelburg (21).

Not only has Kruck endeavored to answer this question in the former sense, for Ekambaram has also argued that this is the correct view, and has been at some pains to explicate the structure of the door mechanism and "sensitive hairs", but I think, incorrectly. Other and reasoned argument I know not of, while, on the other side of the question, as said above, both Merl and Czaja have advanced experimental evidence. Thus we are faced again with the alternative stated at the close of a previous paragraph.

The Posture of the Door in Relation to the Threshold

By all observers, with the exception of Withycombe (26), the opinion has been held that the free edge of the door rests on the *top* or against the infacing *inner* edge of the threshold, and the figures when supplied leave no doubt about the meaning intended: Cohn (Fig. 1-A), C. Darwin, Dean, Goebel (Fig. 1-B), Meierhofer, Luetzelburg (Figs. 2, 3), Brocher (Fig. 4), Merl, Czaja (Fig. 5), Ekambaram. To the earlier, in whose opinion the door was a mere passive

*The properties of the wall have been extensively studied by Czaja (6, 7).

valve, there appeared nothing more to say. After Brocher made his important observation, however, it became necessary to account for the water-tightness of the door. Brocher figured a blob of mucilage filling the entrance (Fig. 4) and postulated a compression of the threshold and door by the cramped-in sides of the trap. Others saw in the pressure exerted by the door edge on the top of the threshold (e.g., Czaja, Fig. 5), coupled with mucilage, a sufficient explanation, the supposedly correlated structures of the door being specified. But Withycombe saw that such an explanation is inadequate. Studying

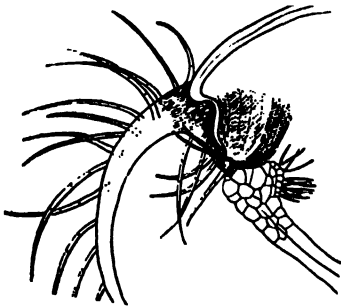


FIG. 3. *U. ochroleuca*.
After Leutzelburg (21).

paraffin sections, he thought that the forward edge of the pavement epithelium affords a resting place for the door edge, resisting its inswing (Fig. 6); he thought, further, to have observed a strand or mass of mucilage attached to the threshold, just in front of the position of the door edge, as postulated by him. While his idea has turned out to be right, his interpretation of the anatomy of the parts involved was incorrect. I have shown (Fig. 7) that the position of the door edge is such that it rests against (not in front of) the pavement epithelium either on a slightly outward-facing surface or (in various species) its mechanical equivalent (Plate IV-40, 41, 42; Fig. 7), that the door edge exerts a downward and inward thrust against a resisting middle zone of the pavement epithelium, and, further, that attached to the forward several courses (the outer zone) of the pavement epithelium there is a membrane, the velum, consisting of the loosened common cuticles of the cells composing it (Plate III, V-47), remaining, however, unloosed from the more forward cells (3 to 5 courses). This velum becomes so adjusted that it folds upward against the outer face of the door edge, thus sealing the slit between the door edge and threshold against inleakage of water*. The velum stretches across the whole front of the downwardly arched threshold as a veil (Plate VI). Since the extreme ends of the free door edge are attached to the inner angle of the threshold ends (c, Figs. 9-13), it follows, as is the fact, that the lateral extensions (a, b, Fig. 17-A) of the free door edge must traverse the threshold

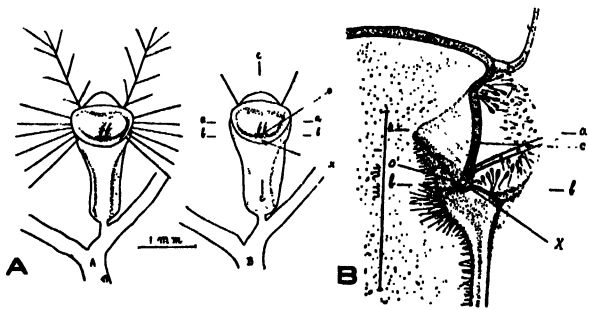


FIG. 4. *U. vulgaris*. After Brocher (1). (A) His drawing recording his observation of the expansion of the walls on "stimulation". (B) The door mechanism.

*I have pointed out elsewhere that a drawing by Giesenhagen (Fig. 8) made at the instance of Professor Goebel, of *U. flexuosa*, showed nearly correctly the velum, no reference to it being made in the text.

to reach the front of the pavement epithelium (Figs. 7, 9, 10, 13, *b,a*). Since the edge of the door which articulates with the wall of the trap articulates also with the ends of the threshold from the inner to the outer angle (*b* to *c*), it further follows that there must be a fold in the door, extending obliquely downward from the outer angle of the threshold (*c*) to the point where the door edge, having crossed the threshold, dips into position in front of the middle zone (*a*). The slit-like space or rather fissure thus formed between the door and the upward-turning threshold is a place where the water, under greater outward pressure, could easily gain entrance into the trap, were it not for the presence of the velum, which completely seals the front of the slit (Lloyd, 18). Thus we have an adequate explanation (*a*) of the ability of the door to resist the superior pressure of water on the outside, and (*b*) of the water-tightness of the door, in spite of the fact that the ends of the free door edge coincide in position with the inner angles of the threshold. Experimental proof will be given anon.

Kruck (17), however, thinks differently. The entrance to the trap, she says, is closed by the door—"dessen schwach vorgebogener freier Rand von innen gegen ein Polster angelehnt ist"—quite the idea of Cohn (Fig. 1) so far as position is concerned. Her drawing accords with this statement (Fig. 14-1). According to this, when the trap is in the normal set condition, the free door edge is so placed that the bristles lie on the threshold, and the edge itself reaches inwardly over the inner margin of the threshold as far as the bifid trichomes. During the transmission of the stimulus which results in the initial opening of the door, the bristles change their position through an angle of about 100° (Fig. 14-2) caused by changes in the curvature of the door, from which, during the 15 min. required for recovery, regression to the original normal position occurs. Admitting the difficulties of direct observation mentioned by Kruck, it is still hard to see how she can have come to her conclusions. In order, however, to oppose objective evidence to hers, rather than merely to criticize, I have studied photographic

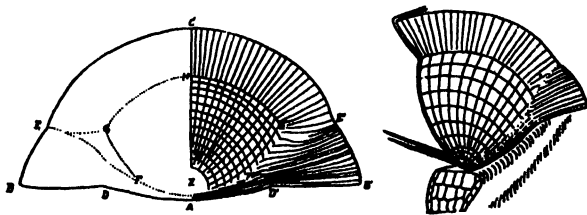


FIG. 5. *U. vulgaris*. After Csaja (5).

silhouettes of traps in various conditions (before and after discharge, etc.) constructing diagrams therefrom. These will be discussed more fully beyond. Here I say only that an inspection of these diagrams, which are not intended to be complete, but only so far as to show the points at issue, namely the position of the door and bristles with respect to the threshold, will at once convince the reader that the free edge of the door cannot possibly reach far enough into the interior of the trap as to permit it to lie against the inside edge of the threshold. As to the exact position of the free door edge, I have already given evidence (18), but the point will stand further elucidation. I take the cases of *U. intermedia* and *U. vulgaris*, not previously dealt with by me in detail. The descriptions of the threshold by Cohn, Hovelaque and

others have no cogency in this connection, for it is quite evident that the minutiae of structure escaped them, aside from other obvious errors of observation. The reason for this can be stated in Cohn's own words. Hitherto various observers "haben natürlich auch nicht vom richtigen Standpunkte die Organisationsverhältnisse aufgefasst".

The Threshold and its Structure in Relation to the Door

The threshold (called the collar by Darwin) is a raised-up arch of tissue, relatively massive, occupying the lower semicircle of the entrance into the trap. Of first importance in understanding its function is to understand the structure of its curved upper surface occupied by the pavement epithelium. If hitherto

thought of any use in the mechanism, the component cells have been allowed to contribute mucilage to the sealing of the door to make it water-tight. It is, however, far more than this. It has become evident to me that the mucilage, undoubtedly present but not peculiar to this region, acts as a lubricator enabling the door, though pressing firmly on the threshold during the opening movement to slip over it with great ease. Beyond this its function is twofold; to provide a firm resting place for the door edge, opposing its inward urge under pressure of the outer water and to furnish, by the exfoliation of the common cuticles of some or all the pavement epithelium cells, the membrane which I

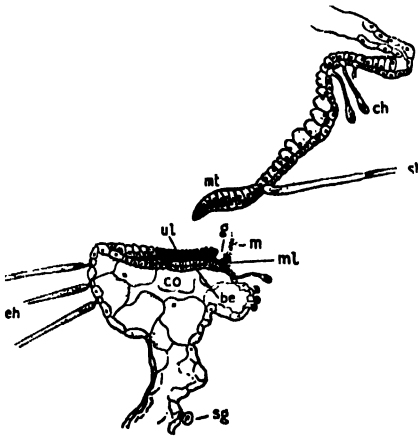


FIG. 6. *U. vulgaris*.
After Withycombe (26)

have called the velum—a veil-like folded-over membrane, firmly attached to the outermost course of cells (Plate VI). In order to see this clearly, one must have recourse preferably to fresh living material, of which suitable sections must be cut through the appropriate planes (see Method). Unless the sections are truly normal to the axis of the threshold, its delicate curvatures are easily masked. When such truly orientated sections are obtained, the pictures afforded are as those seen in the accompanying figures (Plates III and IV). The pavement epithelium is seen to be divisible with respect to the trap as a whole into three transverse zones: inner, middle and outer, the "glandular" cells of which all have the three- or four-celled structure often described from Hovelaque on. The characters of these zones are best seen in a transverse section of the threshold through the middle point (Plate III, IV—40, 41, 42; Fig. 9). The inner zone is composed of loosely packed glandular trichomes with capitals of oval form, one- or chiefly two-celled from between which protrude bristling plates, the remains of the enlarged and autolyzed cuticles. The middle zone is very compact of the capitals, here two-celled, flat-topped and dense of protoplasm and is usually somewhat arched. The outer zone is of looser

bacciliform unicellular capitals to which, for several forward courses, their cuticles remain attached as balloon-like enlargements. To the more forward of these there is attached a broad, irregular-looking membrane, the common cuticles of the middle and inner zones. In such sections as those seen in the figures, this membrane (the velum) appears lax and without orderly position but in a preparation in which the threshold is entire, and properly viewed, the velum rises up in front (Plate II-19) and curls over behind, as we observe in Plate VI-61, 62; III-31. The velum extends throughout the whole length of the threshold, from outer angle to outer angle; a slightly different appearance at the sides (Plate VI-57) being due to the stretching during the development of the trap, for the velum is set free before the full growth of the trap has been reached. It is this, indeed, which causes the velum to take its proper position as a stretched membrane to receive the front of the door edge when the door is normally closed. Otherwise it would be too loose and lax. Further examination of the section will show that

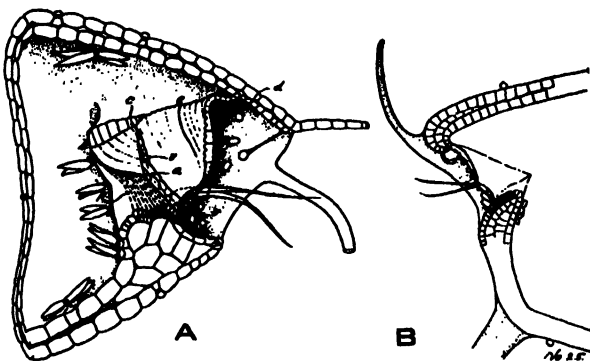


FIG. 7. *U. gibba*. B, *U. reniformis*. After Lloyd (19).

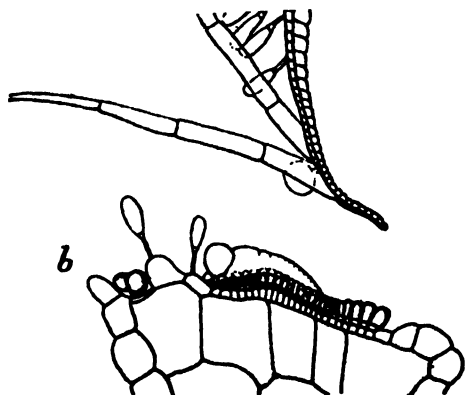


FIG. 8. *U. flexuosa*, showing the velum (b). After Goebel (15).

between the velum and the compact middle zone there is a shallow depression into which the front edge of the middle zone dips down (Plate II-20, IV-40). The resulting forward facing surface is the firm resistant against which the free door edge rests. Without this, the trap could not function as an unstable equilibrium; and without the velum this is equally true. The following is experimental proof of this. (See Plate VI-55, 56, 57, 58). Choosing a well-developed trap, I discharged it by touching the bristles. With a very sharp small scalpel (see

Method) I cut the velum at the side, whereupon the trap walls immediately expanded to their full and remained so, never recovering further exhaustion of the water, nor would the door again act to pressure on the bristles. This experiment I repeated several times. The figures show one example photographed before springing, after this and before operation and a half-hour after the cutting of the velum. Having carefully cut away the door to satisfy myself

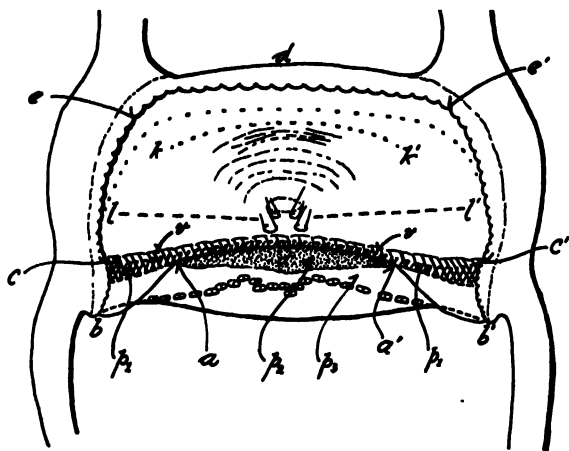


FIG. 9. Door and threshold viewed with axis of vision normal to plane of threshold. *a, a'*, the points at which the door edge leaves the front of the middle zone of pavement epithelium to cross over to the inner angle of threshold; *b, b'*, inner angles of threshold; *c, c'* outer angles of threshold; *e, e'*, points where the upper edge of door turns backward along sides of trap; *l, l'*, line marking change of orientation of outer epidermis cells; *k, k'*, limit between upper hinge and middle zone of door; *d, d'*, median axis of door; *p₁, p₂, p₃*, outer, middle and inner zones of threshold; *v*, velum.

stretch of the threshold, the door edge lies in front of the middle zone, in order to come into this position it must cross the threshold. This it does near the ends (Fig. 9). Here, where this crossing occurs, there is little or no compact zone, but a shallow oblique groove in which the door edge lies is evident (Plate III). Just at the point where the door edge emerges to lie in front, the threshold and its pavement epithelium are rounded transversely and the beginning of the compact zone is seen (Plate II-22). It is interesting to note that the physical opposition of the pavement epithelium as a whole is greater toward the sides (Plate III-26) than in the middle of the door; that is, the middle piece of the door is opposed by a lower obstruction to its inswing than on either side. The instability

that I had not damaged it, or any other part of the mechanism than the velum, I then photographed the latter on the operation side and on the untreated side, to show the tear. The operation is not easy, as the velum is tough and slippery, and it is difficult to hold the trap sufficiently to get the requisite purchase. A second example of a cut velum is shown.

Recurring to the pavement epithelium, we must further note that the zones mentioned are not coterminous with the threshold (Plate II-22). It already has been pointed out that the ends of the free door edge are attached to the inner angles of the threshold, each to each. As, in the middle

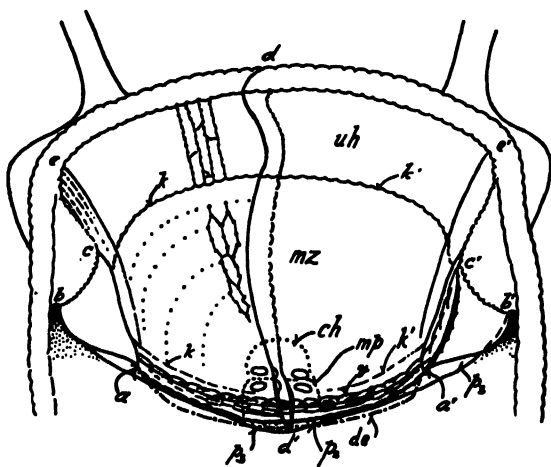


FIG. 10. Same as Fig. 11, but with axis of vision parallel to the plane of threshold. Indices as in Figs. 9 and 17. *uh*, outer hinge; *ms*, middle zone; *ch*, central hinge; *mp*, middle piece (of door); *de*, edge of middle scallop of door lying a little below level of *p₂*. From *c'* to *a'*, the broad dark line indicates the slit between the lateral triangles of door and threshold (exaggerated for visibility).

of the equilibrium is therefore greatest in the middle, precisely where the release of the door edge, by the trigger action of the bristles, takes place. I shall indicate beyond that there is to this purpose a specialized organized portion of the door in the middle reach of the free edge. It is thus that the tripping action of the bristle-door mechanism is a very delicate one, but from experience one may certainly know that "die leiseste Berührung der Haare" (Kruck, 17, p. 260) may not be sufficient. With care, one may often touch the bristles, especially from below upward, without procuring response. But it would be idle to try to state how much pressure is required. I have observed large protozoa crawling about the door and on and about the bristles without any disturbance of the mechanism. Mere touch or slight bending of the tips is not enough. The best we can, indeed all we need to, say is that enough pressure must be given to the bristles to move slightly the middle piece of the door edge, and thus rob it of the even resistance offered by the pavement epithelium.

If the bristles are irritable, it would be reasonable to argue that mere touch regardless of direction would serve as a stimulus. But Czaja noted (1922) that the action ("reaction") of the door is made more difficult when the tension of the trap walls is overhigh; and (b) that the action is achieved more easily by pressure of the bristles downward than sideways, or, he might have added, upwards. I have many times verified the truth of these observations. It is well known that the sensitive hairs of *Aldrovanda* (Czaja, 8) and of *Dionaea* (Brown and Sharp, 2) do not require unidirectional stimulus. Brown and Sharp however found that in *Dionaea* release of the sensitive hair by two sudden partial releases from a previously bent position was unable to procure response; only bending the hair from its normal upright position is effective. It is thus seen that bending the bristles of *Utricularia* in one direction only might alone be effective. But we know that bending in any direction can be effective if in sufficient, though small, amount.

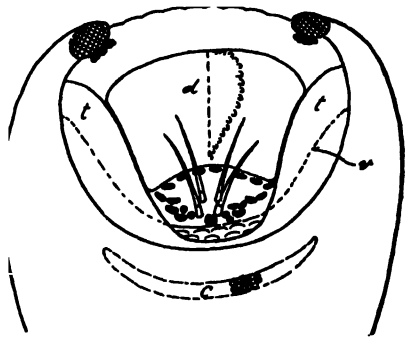


FIG. 11. Door viewed from the front to show the distribution of trichomes on the front of the door (d) and on the sides of the entrance (t); c, zone of sessile trichomes as the edge of entrance (see Fig. 13) *U. intermedia*. Other trichomes in solid black.

Form and Positional Relations of the Door

We may now consider the door with reference to some points of general structure. In view of the several attempts to describe this organ, it would seem here superfluous, were it not for the inaccuracies and inadequacies characterizing most of the presentations. Thus one author described the cells of the inner epidermis of the door as isodiametric, an error, though not an obvious one to a casual observer, as the appearances are deceptive. Kruck described these cells correctly with regard to this point.

In general form, the door when flattened out is roughly semicircular in outline, the free edge lying along the diameter (Figs. 17, 19). In its normal form when not forcibly distorted it is curved and corresponds roughly to a quarter

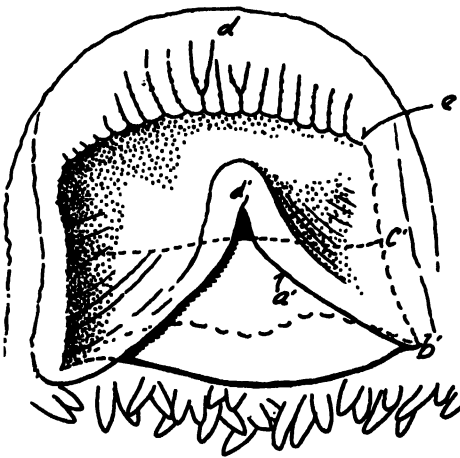


FIG. 12. Diagram taken from a photograph of the door in the act of sliding over the threshold, in position of curve 2, Fig. 18, approximately.

spherical surface (Plate VI-59, Fig. 9) and it is approximately this form which it has when in its position guarding the entrance to the trap (Plate I; IV-38). Its articulation with the wall of the trap is again roughly semicircular, the two ends of the semicircle coinciding with the inner angles of the threshold (Figs. 9, 13). The plane, in which lies the semicircle of attachment, lies at an obtuse angle (*ca.* 100°) with that of the threshold (Fig. 13), being such that the chord of the arc formed by the door edge at its middle point lies near the forward edge of the threshold (Fig. 9). The free door edge is too long to lie on the threshold along its

inner edge and fit it (Fig. 12). The normal position of the door edge is, as above said, with its middle reach against the forward edge of the middle zone of the pavement epithelium* and in order to reach this position the lateral reaches of the door edge must traverse the threshold. It is in this fact that we see the meaning of the shallow scallops characterizing the free door edge seen by everyone, and represented correctly by Czaja (Fig. 5) and by Kruck (Fig. 20). If what I have said be not true, then the curvatures of the door edge have no meaning, but this is far from the case. The middle reach, a low scallop, rests in front of the forwardly, slightly curved middle zone of the pavement epithelium (*a, a'*, Fig. 9); each of two half-scallops (*a b, a' b'*, Fig. 9), or lateral reaches, lies obliquely across the pavement epithelium from the inner angle of the threshold (*b b'*) to a point approximately one-third of the length of the threshold on the forward edge (*a a'*). When the relative positions of door and threshold and their articulation with the wall of the trap are understood in the above sense, it is possible to map out the door into its regions with reference to the play of each during the operation of opening and closing. Czaja attempted this (Fig. 5) but erred in thinking that the outer surface of the door edge is pressed against the top of the pavement epithelium, and plotted an axis of flexure (*E, F, F', E'*, Fig. 5) which does not exist. Kruck's description scarcely departs from that of Czaja and errs so far that she has, *e.g.*, drawn glandular trichomes attached to regions of the outer surface of the door where no trichomes can ever be found, while her drawings of the component cells are completely inadequate (Fig. 20).

*The position of the door edge against the forward edge of the middle zone and housed in the velum, can clearly be seen under a 16 mm. lens if the door is viewed "en face", since they lie close to a single optical plane (Plate II-19).

In Fig. 11 I have supplied a diagram to show the massing of the trichomes on the door and sides of the approach in *U. intermedia* which has a tuft on the forwardly facing surface of the door, much as shown by Goebel (15) for *U. flexuosa*, flanked by two tufts which arise from the walls of the approach and closing access to the spaces formed between the lateral parts of the door and

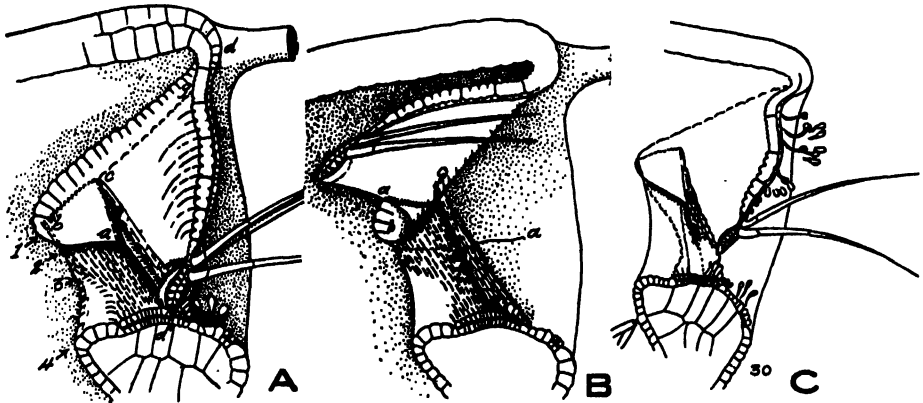


FIG. 13. A and B, diagrams of sagittal section of door apparatus of *U. intermedia*, A, the door in closed position. The position of the middle piece as in curve 1, Fig. 18, is indicated; B, door in open position, approximately. The velum is now lax. C, sagittal section of *U. flexuosa*. The door is raised above its normal position. Drawn from a photograph without readjustment, to show better the velum in lax condition.

trap wall. Together the three tufts form a shallow funnel leading to the base of the bristles, the effect of which is to reflect the errant motions of prey toward the bristles. The drawing made by Skutch (his Fig. 1, 25) shows the relations of these tufts as viewed laterally. The specific structure of the trichomes was detailed by Meierhofer (22).

If we look at the door *in situ* along an axis which passes through the middle of the door at right angles to the plane of the threshold, we can get a true picture of the relation of the door to the trap (Fig. 10). Below we see the contour of the threshold, a somewhat flattened inverted arch, springing from a cushion of cells, one each side (*b a a' b'*). This cushion (just above *c* and *c'*) is the edge of the door. Above appears an arch (*ed e'*) of greater diameter, extending between the bases of the antennae and somewhat beyond. This is the articulation of the middle of the upper edge of the door with the wall of the trap. From the antenna base on each side, the articulation turns backward in a circular

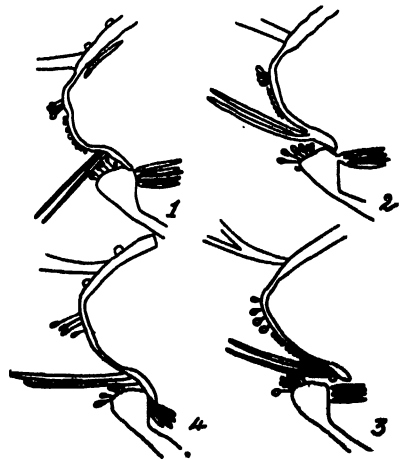


FIG. 14. Diagrams copied from Kruck's figure to show the supposed changes and curvatures of the door during response (Kruck, 17).

sweep to the forward angle of the end of the threshold ($e c b$, Fig. 9). From the forward (c) to the inner angle (b) of the threshold end, the door edge is raised a little away from the wall by the end of the threshold, so that the attached door edge here turns in slightly and, as it crosses the end of the threshold, it turns again to face obliquely the interior of the trap. In effect, the door is cramped in to fit a narrower arc than that which it would, if free, normally occupy, as Brocher apprehended. This makes for a close fitting of the door edge as it traverses the threshold. Luetzelburg (21) showed this detail in his Fig. 47 for *U. ochroleuca*.

If now we dissect away the door, in water, we may map out the areas of significance (Fig. 17-A). When freshly removed, the door retains its curved form, but with more spread, and the outer hinge cells curve more strongly.

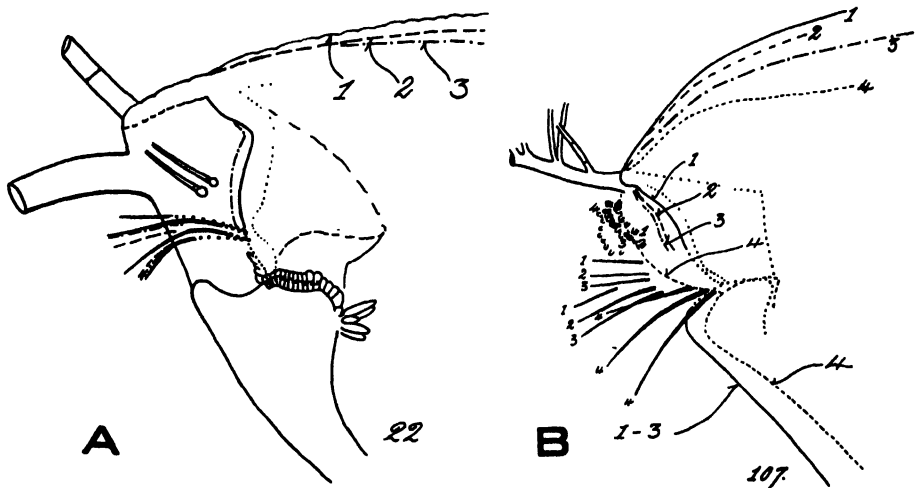


FIG. 15. A, Diagram from projected images of *U. gibba* reproduced as Figs. 1-3, Plate I, to show changes in posture of door and walls; 1, before discharge of the set trap, 2, immediately after, and 3, after puncturing; B, a similar set of diagrams for *U. vulgaris* the additional curve 4 indicating the position of door etc., after making a sagittal section.

This happens immediately and is owing to the turgor pressure of the cells added to the natural elasticity and cramp of the tissues. Plasmolysis with 0.5 *N* potassium nitrate results in flattening them out to some extent, but by no means entirely (Plate II-16). We shall refer to this again. By killing the door with KI-iodine and subjecting it to slight pressure, the door may be sufficiently flattened out for observation. Photography of it is difficult because of the residual curvatures. When flattened, we may arrive at the following analysis. The roughly semicircular form of the door (Fig. 17-A) is bounded along the free door edge ($b b'$) by a curved margin, having the form of an archer's bow of a more ancient sort. The middle scallop ($a a'$) occupies somewhat more than one-third of the length; the end scallops ($a b$, $a' b'$) each less than one-third. The middle scallop lies against the front of the middle zone of the pavement epithelium, itself bowed slightly forward to their mutual fit (Fig. 9). At a and a' , the door edge passes obliquely inwardly across the threshold, so

that the triangle abc lies against the end of the threshold (and mutually $a'b'c'$). The point c lies at the outer angle of the threshold, cb coinciding with the end thereof. The broken line, ac (Fig. 17), indicates a fold in the door, fitting the forward edge of the threshold, from its outer angle to the point where the door edge emerges to the front of the threshold. Thus, from c to a there is a re-entrant space, a mere slit between the face of the door embraced in the triangle abc and the corresponding face of the threshold ($a'c'$, Fig. 10). This is proved by the above-mentioned experiment, in which, by cutting the velum, cc' , which lies in front of this re-entrant angle, water is admitted and the trap is no more able to set itself (Plate VI). This justifies marking of the triangles in question, viz., abc , $a'b'c'$.

Since ce , $c'e'$ are those parts of the door edge which are attached to the lateral walls of the trap, there is a rather gentle fold, ea , $e'a'$ between the lateral and frontal aspects of the door. There is, however, no structural correspondent. That area which remains, ea $e'a'$, is the front-looking part of the door. In this there are two critical areas (a) a roughly circular area, the *central hinge*, the thinnest part of the door just above the bases of the lower pair of bristles; it is a hinge allowing principal movement on the axis, l , l' (Fig. 17-B), and (b) a much thickened part, with very much thickened outer walls, the *middle piece* (Plate VI-59, 60) delimited by the broken line ii' (Fig. 10, 17-A) which rotates backward on l l' (Fig. 17-B) when pressure of the bristles supervenes. This is the tripping action. Beyond the area enclosed by ii' on either side the door edge remains thickened, thinning but little toward b and b' , not having, however, the special strengthening derived from thick outer walls, shown in Figs. 51, 52 of Plate V.

Flexures of the Door during Opening and Closing

We shall better comprehend the histology of the door from the point of view of function if we understand the flexures which it has to undergo during movement. Ekambaram has already told us about this so far as the introverted position is concerned. The door areas are mapped in Fig. 17. First we note that the initial movement which frees the door is a slight upward movement of the middle piece. This can result from any movement of the bristles in any direction, though it is generally conceded that upward movement is least effective. But although upward movement of the bristles is conceded to be not so effective, it

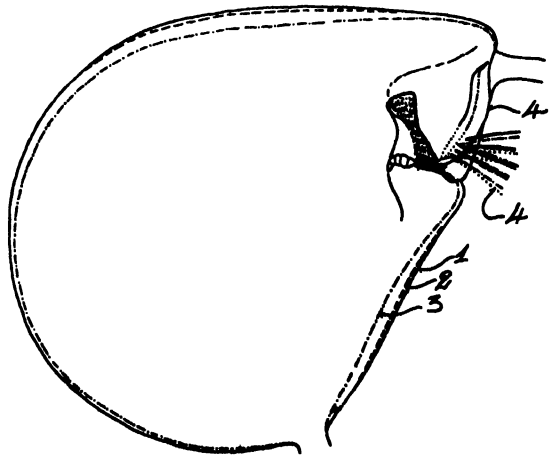


FIG. 16. The same as Fig. 15-B, for *U. intermedia*, from projected images reproduced as Figs. 7-10, Plate I.

does, as a matter of fact, work. We may be correct in explaining this by supposing that the middle piece, being moved slightly outward, releases the door edge on either side, which would be nearly as effective. At all events, the movement must be such as to release part or all of the free edge of the middle piece from its resting place against the middle zone of the pavement epithelium (Figs. 7, 13). This being accomplished, the door is free to move inwardly in front of a column of water which will be great or small according to the extent of exhaustion of water from the interior of the trap. Assuming a maximum, this column of water pressing equally everywhere on the door pushes it inwardly as a whole. Inasmuch as the free door edge is longer than the threshold (Figs. 9, 10, 12) over which it must slide, it must

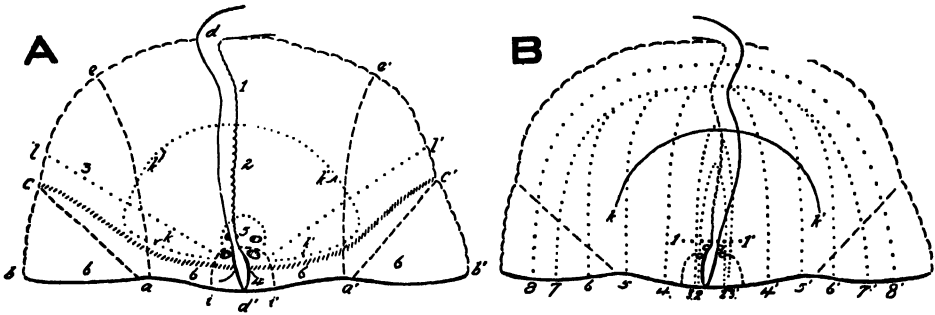


FIG. 17. (cf. Plate II-21). A, plan of door laid out flat, *U. intermedia*. a, angle between middle and lateral scallop of door edge; b, position of inner angle of threshold; c, of outer angle of threshold; d d', median axis of door with outline of sagittal section of door overlaid; e a, approximate position of flexure between front triangle and backward sweeping sides of the door; l to centre to l', line indicating the change of orientation of the outer door epidermis cells from transverse (with respect to radii of door) to longitudinal at the sides (l b i); k k', a closed line inclosing the middle zone (2) and separating it from the outer hinge (1); 5, central hinge; 4, middle piece; 3, identifies the area shown in Fig. 21-A; 6, identifies an area, the edge zone, a part of which is shown in Plate III Fig. 34. This is not a sharply defined area, but is more properly regarded as a continuation of the outer hinge. Primed numbers are mutual with unprimed. The line of the velum edge as it is applied to the door when normally closed is indicated by the hatched line c c' (see Plate II-19 and especially Plate VI-63). B, Diagram to indicate approximately the flexures of the door during its inswing on discharge. 1 1', the axis on which the middle piece swings in clearing the opposing edge of the threshold middle zone to come into position 1, Fig. 18. This is not a constant axis, as touching the bristles laterally, etc., causes flexure of the circular central hinge in the corresponding axis, and this has the same effect. Curve 2 in this Fig. 17-B corresponds to curve 2, Fig. 18, curve 6 to curve 4, and curve 8 to curve 6, approximately.

therefore buckle at some point. This is always at the middle point and nowhere else. By measuring the length of the inner surface of the threshold and the length of the line of the door edge we arrive at the ratio 100 to 110, the threshold being 10% shorter, approximately. Using this measure, the Curve 2, Fig. 18, has been drawn (using a bent wire as a model). To adjust itself to this buckling, the door must curve, this being a matter of observation (Fig. 12). As the edge of the door moves inwardly, the middle piece with its adjacent length of door edge tends to revert to its natural curvature, but since the water column is pushing inwardly, the curvature is reversed and the form of the door will then assume curvatures somewhat as 4 and 5 successively. Since further the water column is now pressing, as if it were a solid rod, on the door radially, the door is opened farther till it assumes the form of Curve 6 in the same

figure; and, as the surface of the door is not that of a true sphere, but is spheroidal, bellying outwardly, it must, during the passage inward, reverse its curvatures, becoming hollowed in the opposite direction from normal. This takes place on passing from Curve 3 to Curve 4, approximately. The introverted position of the door is seen in the now inwardly curved middle zone and middle piece and the position of the latter is such as to swing the bristles up clear of the entrance. They seem to lodge in the tuft of projecting trichomes projecting from the door surface. Ekambaram likened the form of the door at this position to that of a boat, the bristles lying in the hollow like oars. Since he was observing an introverted door which held in that position after some manipulation, it would assume the form of Curve 6a, as I have frequently observed. This cannot be the form of the door as a strong column of water is passing in, especially as the sides of the door have in this position little resistance. A feature of this change of curvature which is not appreciated easily is that which happens because of the sharp change of direction of the upper hinge as it curves back on either side from the transverse reach, as very well seen in *U. gibba* (e', Fig. 12), namely, there is a tendency of the fronting face of the door to work as a unit, namely, *e a a' e'*, Fig. 17, so that along *ea* and *e'a'* there is a maximum of distortion.

Inasmuch as direct observation, because of the swiftness of motion during normal opening, is not possible until slow motion cinematography can be brought to our aid, it is natural to follow the behavior of the door experimentally. This can be done by pushing in the door with a blunt needle; or the door may be made to move by slight lateral pressure on the sides of the trap, this meanwhile lying on its side under observation. An initial pressure of the middle piece is required, possibly a little further pressure to get the door started in the right direction. Doing this one may easily learn that it requires very much more pressure against the belly of the door to push it inward than upon the middle piece. Ekambaram's estimate (11) was obtained by pressure against the middle of the door. This is merely an indication of the amount of pressure the door can withstand when pressed upon locally. When pressed upon by the front of a water column, its effective resistance must be greater. The pressure is distributed outwardly to the edges of the door, tending to make the curve of the hinge zone deeper, and compelling the door edge, especially the middle scallop (*a a'* Fig. 17) to thrust against the pavement epithelium. This thrust must be firm and the door edge must not bend as supposed by Czaja

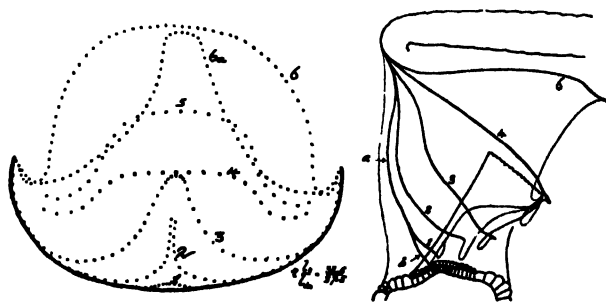


FIG. 18. Diagrams to indicate approximately the curvatures of the door as seen from the front or back, and laterally, the sagittal profile of the door only being indicated. The upward sweep of the middle piece is the first movement (1). Then follows the sharp flexure of the door edge through the middle of the middle piece caused by the greater length of the door edge over that of the threshold. Introversion occurs between 3 and 4.

(Fig. 5) and as implied in other descriptions. This thrust is not shared in by the door edge from b to a and b' to a' , since here the edge is directed inwardly against nothing at all. Instead, the thrust is taken up by the door along the lines $a c$ and $a'c'$, against the pavement epithelium, the curvatures of which conform to this duty (Plate III-26). The whole line of thrust therefore is $c a a' c'$ against the forward edge of the pavement epithelium, but inside the outer velum bearing zone. Obviously $c a$ and $c'a'$ are not straight lines as shown in Fig. 17, but here appear so, due to the exigencies of drawing a flat plan of the door. These two reaches are peculiar in that they are weak places from the point of view of water-tightness of the door, only possible by the presence of the velum, as I shall show beyond.

After the inward swing of the door is complete, as the intruding column of water commences to lag, the intrinsic qualities of the door make themselves felt once more. The position of the door is reversed again, *against the still forward moving water column*. This Brocher, followed by others, showed by puncturing the trap with a needle after it had been sprung in normal fashion. The sides of the trap then still remain concave after normal discharge (Plate II-17, 18; VI-55). This however may not happen if the door has lost its turgor, as then it does not always have a residual elasticity sufficient to the purpose. When a trap dies, the last part to succumb is the door. I have examined supposedly dead traps in numbers and have usually found that, though the walls are quite dead and fully expanded, some of the door cells are still alive. A trap which is in a moribund condition, too much so to exhaust water, may still be made to work by gently pressing out the water which escapes under and around the door edge, but only if care be taken not to push out the door too far so that the velum is caught inside. When the door is dead it can

no longer exercise a strong thrust against the threshold so that when one experiments with such an one, pressing out the water, on gentle release of the pressure the door may give way at once, no snap action being possible. But it is a nice question how far the elasticity of the cell walls of the door alone contribute to the action; for I have noticed that when the door is quite dead, the cell walls have undergone autolysis in the outer hinge zone especially, thus weakening the whole structure.

The total behavior of the door therefore is that of a highly elastic and flexible curved plate, and quite different from what it would be were the door to any extent deprived of these properties, as happens when the door loses turgor.



FIG. 19. Diagram of cell structure of the inner face of door based on original preparation reproduced in Plate II-21. The structure of the central hinge is not indicated; see Fig. 23.

Histology of the Door

It will now be convenient to consider the histology of the door. Its curvatures are simply and better understood by referring in the diagrams (Fig. 17) to the superposed profile of a sagittal section. The effect in a mechanical sense of these curvatures is to offer a convex surface to the hydrostatic pressure greater without than within.

The door is constructed of two courses of cells only, as has long been known, the outer and the inner epidermis. As Meierhofer showed, the door is anatomically a continuation of the wall, itself two cells in thickness, except in specialized regions, *e.g.*, in the threshold and along the vascular tracts.

The outer course is composed of shallow cells with sometimes curved but more often zig-zag lateral walls (*i.e.*, those normal to the surface of the door) and at each angle there is a rod (normal to the surface of the door) (Cohn, 3) giving such support to the lateral walls as to enable the cells to resist collapse under bending (Plate V-43-46; Fig. 21). These cells are capable of only very

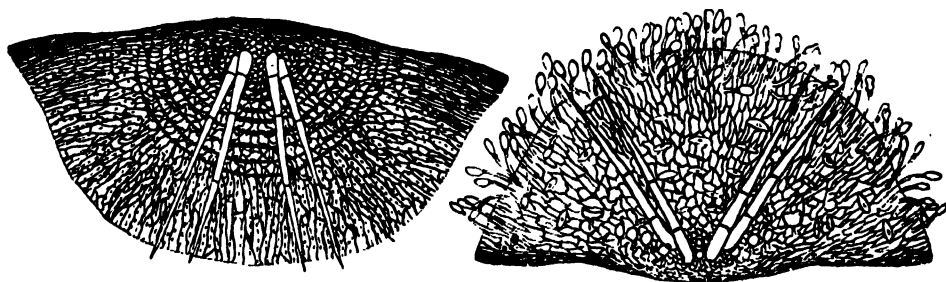


FIG. 20. Inner and outer faces of the door after Kruck.

limited stretching, even under maximum turgor, because of the much greater area of the tangential walls. In the middle triangle of the door there are two regions slightly distinguishable by the character of the shapes of their cells. Those in the outer hinge area are nearly isodiametric and have a zone of cells with a large re-entrant angle formed by curved walls (Fig. 21-B) while those opposite the middle zone are elongated crosswise the door, so that their long axes are normal to the long axes of the cells of the inner course (Fig. 21-C). The pattern of the middle triangle of the door merges laterally with that of the two lateral triangles (lib, Fig. 17-A) where the cells are arranged with the longer axes radially placed, the central hinge being the centre, as Ekambaram showed (Fig. 22). These cells have much simpler and straighter walls, from which angles and rods are absent (Fig. 21-A). In the area covering the middle zone (*h h'* 5, Fig. 17) there is scarcely a trace of curvature in the walls, they being strictly zig-zag. If under the stress of turgor there is a change in the dimensions of these cells, it must be the greater across the door, namely, periclinally in the middle triangle and anticlinally in the lateral. Whether great in amount or not, this stress opposes itself to that in the cells of the inner course to make the door capable of very considerable outward curvature (Plate II-14, 15). As the central hinge is approached, the cells become very small,

nearly isodiametric and relatively more richly supplied with rods, which form an intricate symmetrical pattern (Fig. 23-B). In the middle piece of the door edge the pattern becomes more evidently bilaterally symmetrical, the cells, which have very many and thick rods, gradually merging into the elongated cells of the door edge, which are really part of the lateral triangular regions. Photomicrographs showing small characteristic areas are given as Fig. 43-46, Plate V.

The function of this course of cells, as just briefly indicated, is analogous to that of the metal strip with the lower coefficient of expansion in a metal thermometer. This is shown by the fact that on free bending of the door this layer always tends to take the convex side as occurs especially when a narrow strip from the middle of the door is cut and laid in water (Plate II-14, 15). In any case the amount of extension or of bending of this layer is normally always small.

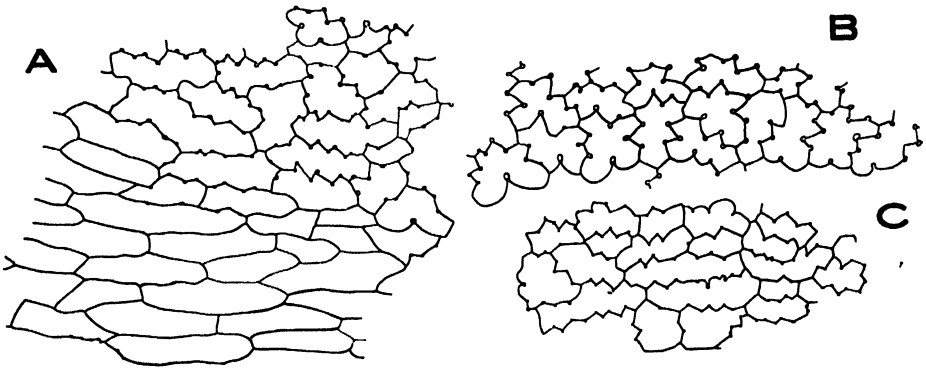


FIG. 21. Outer epidermis of door. A, diagram of cell structure of area near 3, along $l l'$, Fig. 17; B, of area 1, in the same figure and C of area 2 in the same figure. Compare with Plate V-43-46.

The outer surface of the door is always conspicuous for its armature of trichomes (first well described by Meierhofer, 22) of which we are here concerned however only with the bristles or tripping bristles as I have called them. These in the species under consideration are usually four in number, abnormally as many as six (or seven, Darwin) arising as four (resp. more) outer epidermal cells arranged at the angles of a trapezoid which bridges the juncture between the middle piece of the door edge and the centre hinge (Fig. 23). The bristles are gently and elegantly tapering from the base to the extremely tenuous apex and are more or less curved, the lower pair the more. They are normally three-celled, morphologically equivalent to the trichomes in general, though they have been represented by various authors as having four to six cells. The emplacement and spread of the bristles are such as to make them the long arm of a lever with the fulcrum at their base, the weight being represented by the middle piece of the door. (Plate I-5; IV-38; V-51; Figs. 24-A, 24-C).

The inner epidermis is far different. The inner door area consists of the outer hinge (Fig. 17-A, $e k k' e'$ and the lateral extensions), the middle zone ($k k'$), the central hinge (5), the middle piece (4) and the edge zones. The

general arrangement of cells is distinctly radial (Fig. 19; Plates III, IV, V) from a centre in the central hinge. We begin with this. Its cells are very small in all dimensions, but only relatively deeper. The walls are thin, but the large rods serve to support them under the considerable bending they have to suffer; and correlated also with this are the many infolds of the tangential walls, more obviously the outer, in which they occur not only concentric with those of the middle zone, but obliquely, thus adding to the flexibility of the tissue in all directions. As the central hinge merges into the middle piece, in the tissue lying between the upper pair of bristles, the infolds all take a single direction, parallel to the sagittal axis of the door, in conformity with its subjection to the strong longitudinal flexure which takes place when the door is sprung (Fig. 12). The infolds, as of course the rods, are here very numerous and very regularly placed.

In the middle piece the cells are arranged in curves swinging into the edge zone (Plate IV-35) and so on to the outer hinge zone. The pattern is bilaterally symmetrical, merging with the pattern of the outer course of the middle piece which is distinctly bilateral. The very small cells are provided with very numerous plate-like rods lying generally parallel to the line of flexure. The outer walls of these cells are very thick as is the case also of the contingent cells of the inner epidermis.

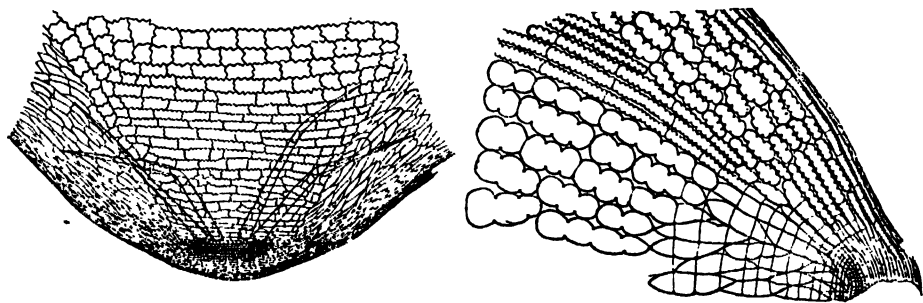


FIG. 22. *Outer and inner epidermis of door after Ekambaram.*

The central hinge is a very small circular area which merges upward and radially with that of the middle zone, the whole radial extent of which is shown in Plate IV-35. Here the cells are much larger and while roughly spindle-shaped (incorrectly described by Czaja as isodiametric), they display much irregularity of form (Fig. 23-A). Their long axes are always radial in position. The side walls, those normal to the surface of the door, are supplied with rods (Goebel, 1891) which occur in periclinal lines, rods which, often cylindrical, may also be irregular plates (Plate V-54) often but not always disposed periclinally. At these the walls usually show "broken joints"—they do not run straight from one to the other side of the rod—this condition often giving rise to quite peculiar appearances. In effect it looks as though the portions of the walls between the rods had been accidentally displaced (Plate IV-35, 39). But the observation which has always pinned the attention is the appearance of circular lines approximately equally spaced, traversing the

long axes of the cells (Fig. 19; Plate IV—35, 37, 39) and centering on the centre of the central hinge. These have been regarded as cell walls (Withycombe, Ekambaram, Czaja) but are not (Goebel, Meierhofer, Kruck). These circular lines have been thought also to be confined to the middle zone, but this again is not true. Here they are more widely spaced radially, are fairly regular, though their regularity has been exaggerated, as e.g., by Kruck (Fig. 20) and convey the impression of concentric circles. In point of fact, the lines are everywhere present but much more numerous, less regular and far less

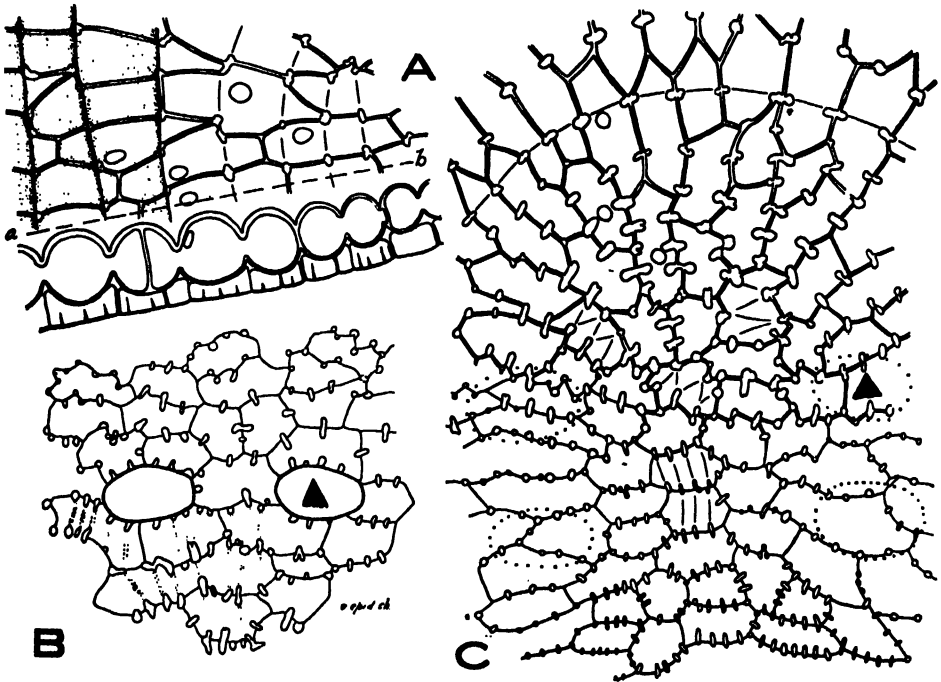


FIG. 23. A. A few of the cells of the inner epidermis of the middle zone to indicate to what is due the appearance of circular lines (see Plate IV—35, 39; Plate V—48). A section running through a-b is shown alongside. To complete the diagram the cells of the outer course are also shown; B, outer epidermis cells in the central hinge (above the bases of the upper pair of bristles) and a portion of the middle piece (below the bristles), cf. Fig. 46, Plate V; C, the inner epidermis of the central hinge and of the middle piece (in part) to be compared with Plate IV—35, 36. In a few of the cells are shown the infolds of the outer walls running hither and yon in the central hinge (i.e., above the dotted-in bases of the upper bristles) and longitudinally (parallel to the sagittal plane) in the middle piece.

conspicuous in the outer hinge zone and in the edge zone. The effect is, in short, produced by a roughly circular *corrugation of the door surface*, to which however there is a corresponding set of corrugations on the inner (tangential) walls of the outer epidermis. Each corrugation runs athwart the radially placed cells without regard to the positions of those cells, but always with regard to the positions of the rods, so that if we plot the rods on a diagram, we have indicated the course of the corrugations.

Regarding a single cell, *en face*, it is roughly spindle-shaped but may have a truncated end (Fig. 23-A). In the lateral walls there are rods which are

placed in pairs one on each side of the cell in the periclinal direction, each one of a pair being the mate of a pair in the neighboring cell, and so on from cell to cell. The outer and inner walls running radially between two pairs of rods are curved like a cylindrical lens, so that running periclinally between the outer ends of each pair of rods there are infolds of the wall (Figs. 23, 24). Since the outer wall is thick and supplied with cuticle, the infold here is optically conspicuous and appears, at an appropriate focus of the microscope, as a bar connecting the tops of the rods. The apparent bar together with the two rods looked to Cohn like ring thickenings, but Meierhofer dissented. Although Kruck realized that a cell wall is not in question, yet thought of the infolds as true bars which, with the rods, form U-bars or U-shaped thickenings.

If now we examine Fig. 24-A we shall see why the prominent circular lines appear as they do. The door is of course more or less curved so that on microscopic examination the optical plane will be parallel to the tops (anticlines) of the corrugations in one place (arrow *a*) but, as the surface of the door is tilted to the eye, the axis of vision will lie as arrow *b*, Fig. 24-A, and when tilted still more the axis of vision will lie as arrow *c*. When the axis of vision is as arrow *a* the image cannot be sharp and as the focal plane is moved up from 1 to 2, the image splits into two ghostly images, sharper on one (the cuticle) side than the other. When looking along axis *b* one looks through a thick layer of the cell wall. It is this which gives us the circular lines. It is true that when looking along axis *a* we can get an image, but it is relatively faint and ill-defined, which always breaks into two symmetrical images on raising the focal plane. There is therefore no structural unit, such as a bar; there is only an infold of the wall. In Plate IV-35, 37, 39 one can see all the various appearances thus obtainable. Various published drawings give the impression that these lines are continuous circles (Fig. 20, *e.g.*) whereas they are never so,

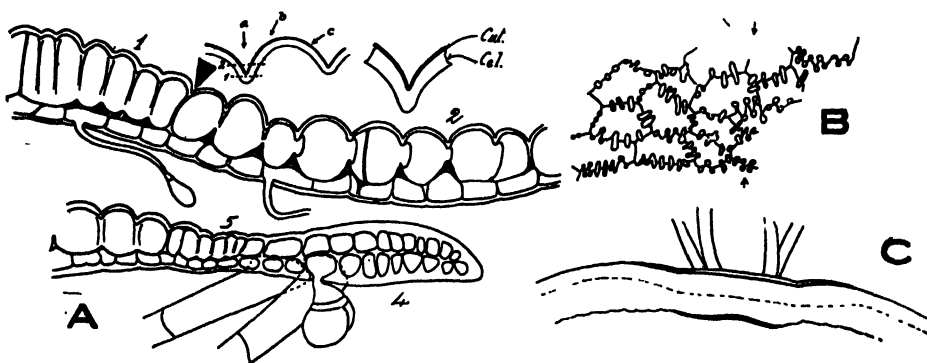


FIG. 24. A, Diagram of longitudinal sagittal section of the door including, at 1, the lower part of outer hinge (ending at pointer), (2), the middle zone, (3), central hinge and (4), middle piece. The numbers along the section refer to the areas indicated in Fig. 17-A. Above: *a*, *b*, and *c* with arrow points, indicate axes of vision which afford various interpretation of the bellows structure; on the left of this, the outer wall to show the cellulose wall *cel.*, with its cuticular investment, *cut.* B, A small area of the outer epidermis of the middle piece near the door edge to show the numerous rods. C, Optical section of the middle part of the door edge to show the middle piece with its thick epidermis and the paired bulbous swellings one on each side of the midline. Compare with the photographs, Plate VI-59, 60.

since they bifurcate and become more numerous as the door edge is approached and even in the middle region they similarly bifurcate.

The total effect of the corrugated inner and outer walls of the cells of the middle zone, as indeed of the hinge zone, is that of a bellows, thus appropriately called by Ekambaram (1916). The inner epidermis therefore is capable not only of being twisted but of much expansion and compression, the former being normally active, the latter passive. The tendency to expansion is ever present in the living door to bring it into its proper position when, after tripping, the door has been pushed in by the pressing column of water.

The outer hinge zone (between $k k'$ and $b d b'$, Fig. 17-A) which occupies a width of about one-third of the longitudinal depth of the door, widens suddenly as it approaches the door edge, where it merges with a zone, the edge zone (Fig. 17-A, 6) having about the depth of the middle piece. The histological character of the edge zone is like that of the outer hinge zone (Fig. 19) and they will be considered together. Here the cells are longer and have more nearly transverse septa but in the edge zone, oblique; there are many more and more closely placed rods and correlatively more infolds of the outer wall. These cells exhibit the maximum capacity of bending or stretching the outer walls (Plate II-14, 15) and can turn the door outward through a wide angle (about 120 to 140°). The cylindrically curved sections or bellows structure of the outer and inner tangential walls are principally concerned with this property (Fig. 26).

The lateral walls of all these cells are flat and run straight between rod and rod. From bay to bay the direction may change somewhat, so that the radial lateral cell walls are variously placed within limits. The small amount of stretching normally imposed on these walls would be easily taken up by them. I am unable to confirm Kruck in stating that between the cells there are pectin masses.

Some published drawings of these cells show them with strongly curved, spring-like *lateral* walls between the rods (Ekambaram), while other observers having seen them, speak of such walls (Fig. 22). This is a mistake. They appear thus in the door when it *has been removed and allowed to lie in water*, especially when flattened out for observation. Soon, near the cut or torn edges, those entire cells next the cut or tear take up water and bulge laterally, at the expense of and encroaching on their (damaged) neighbors (Plate IV-35) and in a short while the disturbance spreads till an entirely false picture obtains. If a door is removed and photographed at once, before the cells have had opportunity to take up water (or better still, *in situ*) the walls are all nearly straight (slight curvatures only may be seen), except at the ends of the entire cells next or near the cut edge. If the door be immediately plasmolyzed or killed with iodine, such bulging does not supervene (Plate II-21; Fig. 19). The walls now, however, are lax, and this equally does not occur in life.

Kruck has stated that the cuticle of the door is not absolutely tightly applied. To this I cannot subscribe. Sometimes when a preparation lies in water, the cell walls of the door become rapidly hydrolyzed and the cells consequently completely disorganized, setting the cuticle free. Cases of this kind offer in

my experience the only suggestion of what Kruck may have seen. There is, in fact, indicatory evidence that the cellulose of the walls of the door cells is different from that of the walls in general (Lloyd, 19) though at the moment I can do no more than offer the above evidence, to which, however, may be added that the extremely elastic character of the door suggests that the cellulose is of the nature of that of collenchyma. Preparations stained with Sudan III demonstrate a tightly coherent, delicate cuticle everywhere on the door surface. Sulphuric acid sets it free.

Summarizing the above, it becomes evident that the door is, as generally stated, a strongly elastic structure, bending readily not only along its radial "static" lines (Czaja) but in any direction. In a condition of turgor the tissue exerts its maximum bending outwardly, never inwardly, with respect to the trap interior (Plate II—14—16). This maximum bending results from the strains inherent in the cell-walls, plus the turgor pressure. When plasmolyzed, thus subtracting the turgor pressure, the door *still bends outwardly*, though not as much, making now an angle of about 45°, with its enforced position when attached normally. The inherent curvatures are owing to the fact that the extensibility of the outer door epidermis is less than that of the inner epidermis with its bellows structure. The construction is such that the door continually strives to take a position outwardly beyond that which, by virtue of a *force majeure*, it is compelled to take; that, namely, in the living, uninjured trap. It can take no other position except as the pressure of the outer water affects it on tripping the trigger mechanism, which is the bristles and thickened middle piece of the door edge. The mobility of the door is the result of the bellows-shaped inner and outer walls of the inner epidermis.

The Door Regarded as an Irritable Mechanism

We may now consider specifically that view advanced recently by Kruck, earlier by Ekambaram, and examined by Merl experimentally. Merl concluded against the idea that we are here dealing with an irritable mechanism and Czaja agreed. Ekambaram found evidence in the structure of the "six-celled" bristles, and in the presence amongst them of short cells, analogous to those in the sensitive hairs of *Aldrovanda*. But the bristles are normally three-celled, and are morphologically equivalent to the three-celled glandular trichomes. The small, so-called, hinge cell does not exist. I have examined, among other species, *U. flexuosa* (Fig. 13-C) with which Ekambaram worked. Merl, on the other hand, determined the limiting temperatures, etc., which affect the mechanism getting only a negative answer to the question of irritability. Kruck's argument and supporting evidence has something of challenge in it. It is as follows.

She fixed the trap in position in a bed of agar, so to support it that she could look directly at the door with a suitable objective. Beginning with a set trap, she first made camera lucida drawings of a few cells of the hinge and middle zones (Ansatz, Mittelstück). The bristles were then stimulated by means of a hair, and the hair was then inserted under the free door edge (thus holding the door somewhat open). The same cells as before were now drawn again.

and there appeared distinct differences in the two conditions. During the irritable condition the cells were shorter, broader and had strongly curved walls. The intercellular spaces, especially in the earlier condition, appeared partly very large and filled with air. During the "swallowing" action, the cells elongated, became narrower, and, for the most part, showed no intercellular spaces (presumably the air had been displaced or changed in its shapes). They had become much more translucent in the event that they were still present. After 15 min., the first condition was "almost" completely restored. So much for observation.

The result of this irritable phenomenon Kruck describes thus (Fig. 14). The outer course of cells remains the same, as also the inner in the anticlinal direction. But in the radial direction there is a straightening of the curvature and hence a lengthening of the door. At the same time, the door becomes more lax and gives inwardly, but not so easily outwardly, because here the turgescient cells are. The changes depend on the form of the walls, determined by the thickenings; they are stretched by turgor and become lax by its reduction.

In the hinge zone (Ansatzzone), the cells could be studied as above. In the unstimulated condition they are shorter, the rods (Stifte) closer together, but when stimulated they are longer. In the anticlinal direction the size is not influenced by the broadening of the cells, but the intercellulars and the pectin yield. These cells constitute a hinge which compensates the movement of the middle zone, but at the same time, when at rest, procures a forward pushing of the same. Under stimulation, on the contrary, it draws it back (inwardly?) somewhat. The zones between the [free] edge and the middle piece are of like structure, but there is effected a broadening through tension just as at the borders of the hinge cells.

Basing her interpretation of the movement of the door on the preceding statements, she proceeds to describe its mechanism. The edge of the door in its unstimulated condition is broad and is drawn back, and, as it were, appressed [against the threshold] by the vaulting of the middle zone. The opening itself is rendered firm by the ring of rigid tissue (threshold). The door and its edge are mutually appressed and rendered rigid by turgescence. The middle zone, equally turgor rigid, is strongly domed in the anticlinal direction and rendered rigid by the studs or rods*. The free edge is difficultly bendable because of its stiff cells. The hinge zone, also stiff, is drawn back in the middle and pushed forward at the edge. In the bristle area, the cells are turgescient, but on account of the rods are broadened but not elevated. The

*In most descriptions much stress is laid on the stiffness of the door, this being attributed to the rods, turgor. Without denying the stiffness, without which it would be no good at all, it seems rather more pertinent to emphasize rather the great flexibility of the door, the function of the rods being to insure the tissue from the crumpling effect of sharp bending which otherwise would cause a collapse of the lateral walls. The rigidity of the door is more than enough to withstand the hydrostatic pressure of the outer medium when equally applied everywhere against a convex surface. Ekambaram (1916) made an interesting rough measure of the resistance of the door to pressure and found that, with the apparatus he used, a pressure of 250 mg. was required to push in the door with a blunt needle. Of course, as he realized, this is not a true measure of the resistance of the door to water pressing equally everywhere.

bristles exhibit a tension of the membranes at the base. What happens on stimulation now follows.

If the bristles are touched the protoplasts contract by giving up water. The tension set up at their bases drags on the plasmodesms and on the walls. The stimulus is transferred to the area about the bristle bases. These cells contract and the door edge draws back "lightning quickly", thereby setting the door slightly open at the edge (Fig. 14-2). The tension of the walls now draws in water. But the middle zone has not yet received the stimulus, and the opening remains yet very narrow.

The stimulus now spreads to the marginal zone, the "Indifferenzzone", and the hinge piece draws back the door edge by the narrowing of its cells. The swallowing (movement) becomes stronger because the middle zone becomes lax and easily bent inward, and at the same time becomes less bowed (domed) and lengthens the door outwardly. The hinge cells become lax and by stretching permit the door to bend inwardly. After the swallowing movement has been completed, the door is pushed against the opening from within (Fig. 14-4). The opening is now closed again and exhaustion of the water by the walls begins. (One would suppose that until the set position is again achieved, which takes "15 minutes", exhaustion of the water could not proceed, since the door is lax.) But the door is not yet irritable, which happens only when the hinge piece is shortened in the middle, the door has bowed itself and the edge stretched forward.

I have been at pains to transcribe the details of Kruck's account; for the rather obvious reason that her view calls for an extraordinarily complex series of behaviors. By way of summary it may be briefly stated that (a) the bristles on receiving a stimulus, contract their protoplasts, expelling water; (b) the stimulus is transferred to the cells of the door immediately surrounding the bases of the bristles, procuring a slight contraction of the door and a release of the door edge, allowing a little water to pass in (under the draught of the trap walls); (c) the stimulus is transferred to the vaulted middle piece and finally to the hinge zone, when the walls of the trap can exert their full draught; (d) after the act of "swallowing" the restitution of the original irritable condition is brought about by a reversal of the whole process, this occupying about 15 min. Kruck postulates changes in the form of the door which accompany the above transference of stimulus, owing to changes in turgor of the cells.

Against the View that the Door is Irritable

In meeting point for point the essential features of this account, we shall first consider if changes in the form and posture of the door occur. To answer this question I have photographed several traps of three species. *U. gibba*, *U. intermedia* and *U. vulgaris*, (a) during the "irritable" state, or, as I would prefer to say, during the set condition; (b) immediately after "stimulation" or tripping, by touching the bristles; (c) after puncturing the wall with a fine needle; and (d) after splitting the same trap along the sagittal plane. These photographs were made very carefully, having regard to the constant orientation of the trap, no lateral pressure being allowed (Plate I; Plate IV-38;

Figs. 7, 13, 15, 16). Diapositives being made, the images were thrown on a screen and tracings made, either of the whole trap, including the door, or merely of the door portion. Only the details which could be clearly seen were traced, namely, so much of the profile of the door as was clearly visible, and the bristles and other outlines, such as the bases of the antennae, the forward edge of the threshold, and certain translucent parts whose relations could not be made out clearly till a sagittal section was examined. I reproduce one such sketch for each species, the details which were clearly visible being duly indicated and distinguishable from the remaining structures. Inspection of the diagrams (Figs. 15, 16) will indicate that the major portion of the outer door surface is visible, as also the bristles, except sometimes quite near their bases.

Specifically, by comparing the photographs as above indicated (Figs. 15, 16), we learn (a) that the convexity of the door surface and its total posture is changed very little after tripping. They are indeed so nearly alike that with the eye alone it is usually impossible to observe any difference. That there is, however, a slight difference in posture is indicated by a slight change in position of the bristles*, some of which may be only apparent owing to slight differences in position of the trap in successive photographs. That some small difference may occur because of the reduced water pressure on the door is only to be expected, but it is difficultly measurable. This appears true also from the fact that when the trap is punctured, thus equalizing the water pressure within and without, the door moves quite measurably farther forward, but the curvature of the door changes but very slightly and chiefly in the region of the hinge (Plate I). If now the derived outlines be compared with a sagittal section and the outlines of the door section and that of the threshold be plotted in, it will be seen that by no stretch of the imagination could we suppose the free door edge to be placed against the inner margin of the threshold, as Kruck has it. It is quite possible to confirm this by making photographs of the door *en face*, both from without and from within, as we have learnt that a total release of the tensions alters the position of the door very little. Taking the front view, from the outside, of a door in an uninjured and set trap, we find that the velum and door edge lie in almost the same optical plane (Plate II-19), such optical condition being impossible if the door edge lay behind the threshold. Cutting the door so that it is not held back by its lateral parts, it swings forward, as every part would, if free (Plate II-14, 15). The above behaviors of the door are easily understandable on the assumption, which I believe to accord with the fact, that the door is simply elastic, and striving by outward movement to take a form from which it is constrained by its attachments. This is not to say, however, that the door is not an irritable system. It conceivably might be and still show the behaviors noted. The evidence, however, is directly opposed to the account given by Kruck and has the advantage of direct and recordable observation.

It will be noted by examination of the diagrams (Figs. 15, 16) that the profile of the trap as a whole changes, as the result of expansion of the lateral walls.

* The proper position of the door as indicated by that of the bristles was clearly understood by Withycombe (26). See his Fig. 1, p. 402.

The pressure of water within and without being equalized, after puncturing, the door is moved forward by its own turgor and elasticity, so far as it may be allowed by the velum. There is then a slightly greater curvature at the centre hinge, altering the position of the bristles somewhat. This is very apparent in Fig. 15-B, position 4.

Merl argued that if the *Utricularia* trap is an irritable system, changes in the form and distribution of air in the intercellular spaces in the side walls should follow stimulation, but he found none. Kruck used the same argument, directing her attention, as we have seen, specifically to the air spaces in the intercellulars of the door. These occur between the outer and inner courses of cells but are confined usually to the upper part of the frontal triangle $e k k' e'$ (Fig. 17-A). She claims to have seen such changes within the limits of observability and measurability, following stimulation. She argues therefrom that the cells must have exuded water into the spaces, evidence for irritability.

I have repeated Kruck's experiment, which consisted in observing the door before and after stimulation, the trap being held in suitable position in a bed of agar. It is important to note that in Kruck's experiment, during the observation of the door following stimulation, the door edge was supported by a hair pushed underneath. It is an assumption which may not be justified that this treatment does not disturb the relation of the door curvatures. Instead of a hair I used a very thin wedge of paper which would produce much more distortion of the tissues. Photographs were taken before stimulation, within one minute after stimulation, and in some cases after puncturing the wall with a fine needle. These are reproduced in Figs. 11-13, Plate II. The experiment was several times repeated, and on no occasion could the slightest difference in the distribution of air, or in the shape of the bubbles, be observed. This evidence is perfectly convincing and denies that there is any exudation of water into the intercellular spaces after stimulation, or even after puncturing which procures maximum relaxation of the door. Even this evidence again does not exclude the possibility of irritability, since, if exudation does occur, the amount of contraction of the cell walls, following reduction of volume, would enlarge the intercellular spaces in equal amount. Thus the exuded water might be accommodated without measurable changes in the included air bubbles. We should expect some change, to be sure, but, supposing we should be wrong, we may approach the matter more directly.

Kruck states that the stimulus is received by the bristles, the protoplasts of which contract, the stimulus being thus transferred to the cells about their bases and so on toward the hinge zone. The door surface has been conceded to be insensitive. What now should happen were we to kill the bristles and door with a suitable toxic agent, leaving the walls unaffected? This experiment I did by irrigating the door with strong KI-iodine, holding the set trap in such position that the iodine drained away without touching the walls, or at least without reaching them sufficiently to denature them within the time required for the treatment. I thus irrigated the door until all the trichomes were stained a deep brown, and I have observed in other material that the iodine penetrates very quickly through the cuticle, though some stains (e.g., Ruthenium Red)

do not penetrate at all, and must be allowed to diffuse through the exposed cell surfaces at the torn edge of a dissected-away door. After the trichomes were completely killed and probably also the door cells about the ends of the bristles, if not all (of the extent of the killing we cannot be completely sure), the bristles were touched with a needle and the usual response was obtained. I repeated this experiment a dozen times and recorded one case photographically (Plate II-17, 18). I may remark that it is not quite so easy to spring the trap after such treatment, since the bristles are then not so stiff, having lost their turgor. One must touch them near the bases, avoiding touching the surface of the door (since this is an *ad hoc* experiment). I have already pointed out that the elasticity of the door is a function of turgor and of the physical properties of the cellulose in its conformation peculiar to the door. My experience thus accords with that of Withycombe (26, p. 410) who observed that traps which had lain a half-hour in Bouin's picroformol but which had not been "fired", retaining their set condition, sometimes were discharged by rough handling. He observed that he could not believe the traps to have remained alive (but in spite of the significance of this evidence persisted in regarding the door as an irritable system).

According to Kruck, following a stimulation, the door cannot respond again for a period of 15 min. Merl of course had shown that the set condition can be regained in this period merely because it requires this as a minimum for an exhaustion of water from the interior sufficient to actuate the door on tripping. The following experiment seems to show that Kruck is mistaken. If, after discharging a trap while lying on its side in a film of water, the sides of the trap are carefully compressed with the side of a needle, it is possible to discharge sufficient water to reset the trap. This is not easy, as a very little too much pressure can easily damage its walls. But it can be done. Numerous times I have achieved the discharge of the trap twice (the original and repeat) within a half-minute; sometimes even eight repeats within five minutes, so that it is possible to discharge a trap nine or even more times in about three minutes. If the door is an irritable mechanism, the rest period required is scarcely more than 15 sec. It seems, however, a fair inference that the door is not irritable, but is merely a physical mechanism.

Ekambaram (1916) did this experiment, after some preliminary manipulation consisting of pressing the trap with a pair of pincers. On the release of pressure, though water did not enter the trap, air escaped into it from the intercellular spaces. After this was repeated "twice or thrice", the trap assumed the "hungry" condition, that is, the walls remained collapsed, insuring reduced internal water pressure. Then the bristles were touched and the trap was discharged, this being repeated thrice. Aside from the difficulty of seeing how the intercellular air could have entered the interior unless the cuticle was broken (I have repeated the experiment *ad hoc* but found no entrance of the intercellular air into the trap), Ekambaram's experience coincides with mine, save that I did not find it necessary to undertake the preliminaries. But this author nevertheless holds the view that the mechanism is irritable. In spite of his declaration that "The motor tissues are not confined to the immediate

vicinity of the irritable (*sic*) hairs (that is the bristles) but comprise the collar, valve and the tissue attaching the valve to the orifice of the bladder" he was not able to stimulate the mechanism to action by touching any of these parts, but only when the bristles were "stimulated". It will be recalled that touching the surface of the leaf in *Aldrovanda* (Czaja) and in *Dionaea* (Brown and Sharp) stimulates as well as touching the sensitive hairs, so that, if it is as claimed by Ekambaram and by Kruck, the result is unexpected and unique. But I have already pointed out the common experience of failure to procure action of the door by bending the bristles through no fault in the mechanism, which could be discharged by a heavier touch. The real implication of Ekambaram's experiment escaped him.

It is further pertinent to consider the time relations of the door action. Every observer, from Darwin down, agrees that it is very rapid. On touching the bristles, immediately the sides of the trap bulge, but the door movement is so sudden as to escape the eye. It is very much more rapid than the reaction of the leaf lobes of *Aldrovanda*, though this is among the most rapid movements of the kind known. Kruck concedes that the initial slight withdrawal of the door edge is very rapid ("blitzschnell") but gives us no idea what time the rest of the reaction, namely the spreading of the stimulus to the hinge zone, requires. Moreover, water is said to commence to flow in on the completion of the initial response; this one might argue would nullify the proper effect, which is a rapid movement of a column of water drawn in vigorously by expanding walls. It happens that I have been able to measure the maximum limit of time required by means of moving pictures, and the record shows that the whole movement occupies less than one-sixteenth of a second, the action itself always escaping the camera moving at normal rate, 16 exposures per second. Though the mere rapidity of action is no final argument against the view held by Kruck, it is nevertheless evidence which makes the need of enquiry the more insistent.

A further argument may be drawn from the total movement of the door after discharge, if the evidence in regard to its posture and flexures already advanced may be depended upon. The door, during the period of unstable equilibrium rests with a downward and inward thrust against the forwardly tilted front edge of the middle zone of the pavement epithelium. The release from this position can occur, according to the protagonists of the theory of irritability, only by a change in turgor altering the dimensions of the door one way or the other—shortening it longitudinally (Brocher, Withycombe) or lengthening it by altering the curvatures (Kruck)—which is now the condition that, to be reversed so that the door may regain its irritability, requires a rest period, or rather period of recovery, of at least 15 min. Nevertheless, after discharge the door *immediately falls back into its original form and position*. All except Kruck admit this, against whom the force of this argument is lost unless the premise established by my experiment on posture be accepted. This means either that the recovery of the original turgor conditions must be as rapid as the loss, each phase occupying not more than one-thirty-second of a second, since the whole movement takes less than one-sixteenth of a second, or the door does

not return at once to its original water-tight position and therefore the reduction of water pressure within the trap cannot commence till it does. But Kruck says that the set condition is acquired in 15 min., the changes in the door occupying this period; but a sufficient exhaustion of water by the walls of the trap to reset it also takes a minimum of 15 min. (Merl, Czaja) as I have very many times found myself. Either therefore the exhaustion of the water must go on simultaneously with the recovery of turgor by the door, which is impossible, as Euclid would say, or the exhaustion of water must begin after the door is once again sealed, which would mean 30 min. for the whole process, which is contrary to fact.

An Argument from Comparative Study

During the past three years I have studied material alive or preserved of some 75 species of *Utricularia*. This material I have obtained by the cordial co-operation of colleagues in various parts of the world. Their help I have duly acknowledged in a paper just issued (20). On the basis of the study of their door mechanisms, the species fall into the following groups, presented in tabular form, each group or category being placed under an index species. The numbers are the numbers of my collection.

TABLE I

THE SPECIES OF *Utricularia* (INCL. *Polypompholyx* AS IF *Utricularia*) GROUPED ACCORDING TO THE CATEGORY TO WHICH THEY HAVE BEEN REFERRED.

DUPLICATE MATERIAL IS INDICATED BY ITALICS

		Total
Polypompholyx	53, 66, 100, 104	2
Hookeri	52, 57, 82, 97, 102, 103, 105, 98, 101	5
Caerulea	6, 8, 11, 31, 32, 35, 37, 40, 55, 59, 79, 81, 85, 91, 94, 95	9
Orbiculata	33, 34, 65, 93	3
Globulariaefolia	16, 45, 62, 63, 76, 78, 96	4
Capensis	1, 4, 5, 48, 84	5
Reniformis	7, 9, 10, 17, 19, 24, 25, 26, 36, 44, 46(?), 50, 56, 58, 61, 64, 75, 77, 79	15
Subulata	3, 18, 20, 21, 46(?), 49, 70, 71, 74, 89, 90	8
Kirkii	5	1
Vulgaris	2, 15, 23, 28, 29, 30, 38, 42, 67, 87, 88, 92	9
Gibba	13, 22, 27, 39, 41, 51, 54, 60, 72, 86	8
Purpurea	14, 47, 68, 69, 80, 99	4
Cornuta	12, 43	1
Lateriflora	83	1
Longiciliata	73	1
		76
	Corr. 1	75

Much of what I might say about these categories has already been said in my Flora paper (20) of this year, I shall therefore indicate only briefly the nature of the argument which arises out of their study. For fuller details see papers Nos. 18-20.

Polypompholyx

The evidence, in the absence of the study of living material, indicates that, in this genus (once regarded as *Utricularia*) the door acts as a valve, in the

sense of Charles Darwin. This view is based on the fact that there is no localized hinge mechanism, either outer hinge or central hinge, and no such localization of areas seen in *U. vulgaris*. The free edge of the door rests on the threshold with a slight overhang on the inside, with no surface resistant to the door edge. There is no extensive velum and no specialized zonation of the very meagre pavement epithelium. But the histology characteristic of the door as a pliable, elastic body is present; rods, infolds, (these do not bulk large in the structure): the latter are irregular, the former slender. In a word the structure is as if the door were all hinge; there being no clear zonation of cells. The outer surface of the door has only low, sessile glands, few in number, and no bristles. The walls of the trap are thick (four courses of cells) and form a triangular structure, as seen in the transverse section, and there seems to be no exhaustion of water therefrom. The trap is, I believe, not capable of setting, in the sense of *U. vulgaris*; or one may say is always set. This judgment is in the absence of the study of the living material and is open to amendment. But the evidence indicates absence of anything but passive movement, yet it has a fundamental histology in common with *Utricularia*, save in those features connected with the nice adjustment of door and threshold to resist water pressure and inleakage.

U. hookeri

This is a type of a number of species all Australasian, in which the door rests on the threshold, somewhat as in *Polypompholyx*, and there is no surface of resistance of the threshold for the door edge. The door rests on an up-turned angle of the threshold but at this point there is a sharp angle of permanent downward (outward) flexure in the door, this being applied to the flexure of the threshold. The threshold has a very broad outer zone, which furnishes part of the velum, and a compact middle zone which is sharply bent transversely into a narrow semicircular (nearly) arc into which the door fits so that the angles of the door are attached to the springs of the arc, while the middle of the door edge overhangs. The only trichomes on the door are low and sessile in two groups, one near the flexure and one on the curving face of the door in the centre of the entrance, which is guarded by an extensive, circular velum. This protects the trap against the inleakage of water which otherwise could take place anywhere easily, as there is everywhere a re-entrant fissure between the threshold and the appressed door. While I have not studied the living material I am assured by my genial correspondents, Mr. A. V. Giblin and Mr. Allan McIntyre, of Hobart, Tasmania, that the walls of the living trap are concave. It seems certain that the trap has a snap action which is procured by a longitudinal fold, started by a slight enforced depression on the surface of the door facing the opening in the circular velum, crossing the permanent transverse flexure from which circumstance the snap action is assured. It is difficult to see, if this interpretation is correct, how such snap action could take place on change in turgor postulated by a theory of irritability, since the vigor of the action can only be proportional to the springiness of the door. It should further be noted that the outer hinge is quite thin while

the thickest part of the door is on either side of the transverse flexure, thus pointing to the need for strength at this point, which would be reduced and the door rendered flabby if the turgor were reduced.

This view is strengthened on examination of the details of structure of the door. This has a very large, relatively inflexible middle piece supported on each side by a more flexible area which can fold, and thus allow the flexure upward of the broad middle piece. The inner portion of the threshold is funnel-shaped beyond the flexure, thus accepting a wide free marginal zone of the door and at the same time allowing its side laps to frill on opening and closing.

The whole aspect of the apparatus lends itself readily when understood to a purely mechanical explanation of opening.

The Groups of the Types Caerulea and Cornuta

The simplest and most striking example of this category is *U. cornuta*, which is devoid of antennae or other appendages. The door curves gently downward, its edge resting against the slightly raised-up tissue of the narrow middle zone of the threshold. There are no bristles, and the only glandular trichomes are a few very low sessile ones on the upper exposed half of the door. I have studied this species in the living condition and find that touching the door is insufficient to cause opening; one has to accomplish a localized thrust of considerable force against the exposed curved upper half of the door enough to dent it slightly, and this initial fold upsets the unstable equilibrium. The absence of any bristles or other trichomes which might be regarded as sensitive make it necessary to assume that the surface of the door is sensitive, if the mechanism is irritable at all; but no experimental evidence has been forthcoming that this is the case. On the contrary, it shows clearly the opposite.

The Group of the Type U. globulariaefolia

This group is very homogeneous. A thick walled trap is provided with heavy antennae very thickly armed with long glandular trichomes forming, together with a similar armature of the ramped stalk, a sort of guiding funnel leading to the central region of a small door without bristles and only a very few sessile glandular trichomes. The aspect of the apparatus aligns it with *U. cornuta*, so far as our present argument is concerned. The same may be said of *U. lateriflora*.

The Type U. purpurea

This is one of the two types of floating submersed forms, *U. vulgaris* being of the other. In the former the ventral profile is almost straight and the threshold lies flat instead of being raised from the wall. The door has a very different form from that of *vulgaris*, having no bristles and no other trichomes excepting only a bunch of long, glandular ones with spherical or fusiform capitals, described by Goebel and by Luetzelburg. They spring from a protuberance arising on the middle line of the door, somewhat above the midpoint. Viewed laterally this appears knob shaped, from above crescentic. The outer hinge is all the region above this, and is thin, the effective hinge

being just above the knob. The lower part of the door is quite stiff, but can buckle longitudinally. Its free edge is provided with a thick forwardly turned weal over which rests the velum. The door edge rests on a narrow dished middle zone of the pavement epithelium. A very effective opponent of the door edge is provided by the permanent swollen cuticles of the inner zone (Plate IV—41, 42). Release of the door edge is caused by any slight rotation of the protuberance, so adjusted that such motion lifts the lower part of the door enough to free it from its opponent. The pressure of water of course does the rest. In springing back into position, the door edge slides over the raised-up cuticles, filled with mucilage, with a little click, thus proving that the dimensions of the door have not changed in discharge.

The sensitivity of the traps varies much, but many are quite as much so as those of *U. vulgaris*, since when being lifted out of the water many are sprung by the water film, and swallow air. When lying in the water, sensitive traps may be sprung by a gentle impact on a few, possibly one, of the glandular hairs which radiate out from the knob. It is however significant that frequently one may gently move the hairs about, pushing them here and there without provoking discharge. It is quite certain that merely touching, or even considerable disturbance of the hairs, is not always effective; but in a case of which the hairs may have been variously manipulated, when they are given a sharp albeit gentle thrust with a flat surfaced needle, without touching the knob, discharge may readily follow. Pressure on the knob from above or below with a needle point can cause discharge. When the touch or thrust is effective the response is momentary as in *U. vulgaris*. My honored colleague of former years, Bashford Dean (10), the only one heretofore to have examined this species in the living condition, was wrong when he denied the occurrence of "spasmodic action".

The behavior of *U. purpurea* quite conclusively denies the possibility of explaining it as an irritable response.

U. reniformis and *U. subulata* Groups

All essentially alike and obviously similar to the *U. vulgaris* and *gibba* kinds. The most striking species is *U. lloydii* Merl in MS. of which the traps are dimorphic, one having long external glandular trichomes, the other sessile ones. This observation was first made by my friend Dr. Merl. I found that these two forms of the trap, which occur on the same stolon, differ in the presence on the door of a curious bristle (one only) with a hinge mechanism at its base in one sort of trap (that with short trichomes) while this bristle is absent from the other. Both traps catch prey. It is difficult to harmonize these facts with the irritation theory.

U. longiciliata

This is a unique species, the door of which has but one long very slender trichome of the glandular type jutting out from near the middle point of the door (Merl 24). It is very weak and it is doubtful if it is an effective part of the mechanism; and moreover, in the absence of collateral evidence, might be adduced as suggesting irritability. But the rest of the mechanism aligns itself with the *U. caerulea*-like forms.

U. orbiculata

In this curious plant, there are no bristles, but from the door in front of the entrance there are two sausage-like gelatinous masses which arise from peculiar large trichomes, the capital cells of which burst open, the masses of gelatinous matter straightening out to form the nearly cylindrical strands. If the mechanism is an irritable one, the stimulus must be transmitted through the non-living material.

The U. capensis Group

The trap door is longer and narrower relatively, than in *U. vulgaris*, the upper half being bowed and armed with the tuft of glandular trichomes, the lower half being middle piece save at the sides. At its upper limit there is a single large trichome, the capital cell of which is very large and kriss-shaped, and is folded inwardly between the door and the threshold. The way to this narrow cavity is blocked by numerous trichomes arising from the threshold. I have examined living material and have found the trap to act upon touching the upper rounded half of the door which is composed of thin-walled cells and is easily bent in. The equipment is readily understood as a pure mechanism, which experiment indicates it is.

U. kirkii

A unique species, having a door with a shape somewhat as in *U. capensis*, but the middle piece shorter, and possessed on the inner face of two large tubercles separated by a narrow gully. At their upper limits there are four stiff bristles so adjusted with respect to the threshold and the trichomes forming a funnel-shaped approach thereto, that prey entering the funnel must press on them. The conformation of the door and its relation to the threshold again lend themselves readily to a mechanical explanation as a pure mechanism.

Summary

I have thus briefly indicated that the various mechanisms to be found in a various species of *Utricularia* collectively lend themselves to a purely physical-mechanical explanation. It seems to the writer that when the whole genus (if about one-third of the total number known may be regarded as fairly representative) is surveyed, the inference is that we are here dealing, in the door-threshold apparatus, with purely mechanical "contrivances" all of them conforming to a single general principle, but presenting differences in detail of extraordinary interest. Just as traps, as devised by man, consist of some release mechanism in unstable equilibrium which when tampered with accidentally by a prowling animal nosing the bait snaps and actuates the rest of the trap mechanism, so the door, whether with bristles or without, in some way is possessed of a release mechanism. As such man-made traps have to have a spring or its equivalent, so the traps of *Utricularia* have a source of energy in the out-springly walls, the turgor of whose cells constantly tend to force the walls outward. But, as Brocher first recognized, and as was independently discovered on three other occasions by three different observers, Ekambaram,

Withycombe and Hegner, as Skutch has pointed out, the motive power is usable only because the exhaustion of water from the water-tight trap is accomplished in some way by the walls, that is by their cells, so that the walls collapse. Czaja has called the walls a selective-permeable system, another way of saying the same thing. The trap is therefore far from being "purely passive". This cannot be, as the trap repeatedly sets itself, this being possible only because the door can adjust itself to the entrance and become water-tight. In this the velum is a *sine qua non*, as I have shown experimentally. It acts as a weather stripping, closing the cleft between the door edge and the threshold pavement epithelium and "preventing a draught" (of water). The door itself is complicated and the complications are such as can be understood only if it is postulated that the door has a necessary elasticity and flexibility. To this end the form of the door and of its component cells, and their turgor contribute. A door robbed of its turgor cannot function promptly. Particularly the prompt closing of the door could not fully take place as it does were the turgor of the door reduced, since it closes before the walls are fully, or indeed half, expanded. Even the door therefore is not purely passive, since its effective working depends on its being alive and its cells turgid. But regarded as a mechanism, and assuming its properties, it can be understood in all its details of structure as such. Aside from the general properties of elasticity and flexibility, it is so constructed that it has a definite release or tripping mechanism, the middle piece and its attached bristles (I am speaking now particularly of *U. vulgaris* and its relatives) moving on a special hinge of its own, the central hinge; while the door as a whole has a curved hinge, since its attachment to the trap walls is curved (semi-circular approximately). The structure of the cells in these hinge areas is distinctly correlated with the function of maximum bending and recovery, without which the door could not function at all. The door mechanism is such that it can act as often as there is the occasion of tripping if the walls have performed their duty. The rate at which they can exhaust water is here the limiting factor. About 15 min. after actuation is required for this, but longer is better, as the greater the exhaustion of water, the more pull there is on the inrushing column of water when the door is released. But if we exhaust the trap experimentally, the door will act in normal fashion as long as our patience and skill permit. The answer to the question asked in the title of this discussion is, therefore, in the negative.

Method

A very simple and comfortable method of handling the traps for observation and photography is that of placing them on a bed of cotton wool in water. This allows of easy orientation, holds the trap firmly enough in position, and with a little care the cotton wool does not interfere with the lighting. One spreads over the bottom of a little flat dish a layer of cotton wool and pours on water to cover. The cotton wool should cover the whole bottom, so that it may be anchored by the sides of the vessel. This arrangement permits one to irrigate the door without affecting the walls.

For operations on the trap, I have found the small knives used by ophthalmologists very useful, especially the Ziegler needle for minute operations on the door, etc., and the von Graefe knife for cutting the whole trap. Operations can be carried on with great nicety under a binocular low power microscope, after practice.

For cutting sagittal and other sections I have found it a practical scheme to hold the trap in position on a slide with a triangle of paper, making the cut with a safety razor blade in a mounting (the "Valet"). A short handle is a distinct advantage.

The best method I have found for repeatedly discharging a trap is to lay it on its side and apply pressure with an ophthalmologist's spud on a piece of wet filter paper, which distributes the pressure, under a binocular dissecting microscope.

Appendix

A LIST OF THE SPECIES STUDIED. THE NUMBERS IN TABLE I ARE THOSE IN THIS APPENDIX.

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| 1. <i>U. capensis</i> , Spreng. | 45. <i>U. amethystina</i> , Salzm. |
| 2. <i>U. diploglossa</i> , Web. | 46. <i>U. nana</i> , A. St. Hil. & Girard |
| 3. <i>U. subulata</i> , Linn. | 47. <i>U. elephas</i> , Luetzelburg |
| 4. <i>U. welwitschii</i> , Olivier | 48. <i>U. puberula</i> , Benj. |
| 5. <i>U. kirkii</i> , Stapf | 49. <i>U. pusilla</i> , Vahl. |
| 6. The same as No. 11 | 50. <i>U. geminiloba</i> , Benj. |
| 7. <i>U. reniformis</i> , A. St. Hil. | 51. <i>U. exoleta</i> , R. Br. |
| 8. <i>U. caerulea</i> , Auct. | 52. <i>U. volubilis</i> , R. Br. |
| 9. <i>U. longifolia</i> , Gardn. | 53. <i>Polypompholyx multifida</i> , F. Muell. |
| 10. <i>U. montana</i> , Jacq. | 54. <i>U. neottioides</i> , A. St. Hil. & Girard |
| 11. <i>U. gibbsiae</i> , Stapf | 55. <i>aff. caerulea</i> |
| 12. <i>U. cornuta</i> , Michx. | 56. <i>U. dusenii</i> , Sylvé. |
| 13. <i>U. pallens</i> , A. St. Hil. & Girard | 57. <i>U. menziesii</i> , R. Br. |
| 14. <i>aff. cucullata</i> | 58. <i>U. saudadensis</i> , Merl. |
| 15. <i>U. oligosperma</i> , A. St. Hil. | 59. <i>aff. caerulea</i> |
| 16. <i>U. globulariaefolia</i> , Mart. | 60. <i>U. herzogii</i> , Luetzelburg |
| 17. <i>U. lundii</i> , A. DC. | 61. <i>U. campbelliana</i> , Oliver |
| 18. <i>U. nervosa</i> , G. Weber. | 62. <i>U. amethystina</i> , Salzm. |
| 19. <i>U. reniformis</i> , A. St. Hil. | 63. <i>U. globulariaefolia</i> , Mart. |
| 20. <i>U. subulata</i> , Linn. | 64. <i>U. nelumbifolia</i> , Gardn. |
| 21. <i>U. resupinata</i> , B. D. Greene | 65. <i>U. orbiculata</i> , Wall. |
| 22. <i>U. gibba</i> , Linn. | 66. <i>Polypompholyx multifida</i> , F. Muell. |
| 23. <i>U. vulgaris</i> , Linn. | 67. <i>U. vulgaris</i> , Linn. |
| 24. <i>U. reniformis</i> , A. St. Hil. | 68. <i>U. purpurea</i> , Walt. |
| 25. <i>U. reniformis</i> , A. St. Hil. | 69. <i>U. cucullata</i> , St. Hil. |
| 26. <i>U. endresii</i> , Reichb. | 70. <i>aff. subulata</i> |
| 27. <i>aff. gibba</i> | 71. <i>U. triloba</i> (prob.), Benj. |
| 28. <i>U. intermedia</i> , Hayne | 72. <i>U. neottioides</i> , A. St. Hil. & Girard |
| 28a. <i>U. intermedia</i> , Hayne | 73. <i>U. longiciliata</i> , A. DC. |
| 29. <i>U. stellaris</i> , Linn. | 74. <i>U. subulata</i> , Linn. (or prob. <i>U. nervosa</i> , G. Weber) |
| 30. <i>U. flexuosa</i> , Vahl. | 75. <i>U. dusenii</i> , Sylvé. |
| 31. <i>U. albo-caerulea</i> , Dalz. | 76. <i>U. tridentata</i> (prob.), Sylvé. |
| 32. <i>U. affinis</i> , Wight. | 77. <i>U. lloydii</i> , Merl in MS. |
| 33. <i>U. caerulea</i> , Linn. | 78. <i>U. globulariaefolia</i> , Mart. |
| 34. <i>U. orbiculata</i> , Wall. | 79. <i>U. flaccida</i> , A. DC. |
| 35. <i>U. uliginosa</i> , Vahl. | 80. <i>U. myriocista</i> , A. St. Hil. & Girard |
| 36. <i>U. dusenii</i> , Sylvé. | 81. <i>U. uliginosa</i> , Vahl. |
| 37. <i>U. caerulea</i> , Auct. | 82. <i>U. dichotoma</i> , Labill. |
| 38. <i>U. intermedia</i> , Hayne | 83. <i>U. lateriflora</i> , R. Br. |
| 39. <i>aff. gibba</i> | 84. <i>U. albina</i> , Ride |
| 40. <i>U. caerulea</i> , Linn. | 85. <i>U. bifida</i> , Linn. |
| 41. <i>U. pumila</i> , Walt. | 86. <i>U. exoleta</i> , R. Br. |
| 42. <i>U. mixta</i> | 87. <i>U. flexuosa</i> , Vahl. |
| 43. <i>aff. cornuta</i> | 88. <i>U. geminiscapa</i> , Benj. |
| 44. <i>U. gluckii</i> , Luetz. | |

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| 89. <i>U. subulata</i> forma <i>cleistogama</i> (Gray) Fern. | 97. <i>U. monanthos</i> , Hook. |
| 90. <i>U. resupinata</i> , B. D. Greene | 98. <i>U. violacea</i> , R. Br. |
| 91. <i>aff. caerulea</i> | 99. <i>U. purpurea</i> , Walt. |
| 92. <i>U. flexuosa</i> , Vahl. | 100. <i>Polypompholyx tenella</i> , Lehm. |
| 93. <i>U. striatula</i> , Sm. vel. <i>orbiculata</i> | 101. <i>U. dichotoma</i> , Labill. |
| 94. <i>U. reticulata</i> , Sm. | 102. <i>U. menziesii</i> , R. Br. |
| 95. <i>U. bifida</i> , Linn. | 103. <i>U. hookeri</i> , Lehm. |
| 96. <i>U. modesta</i> , A. DC. | 104. <i>Polypompholyx multifida</i> |
| | 105. <i>U. violacea</i> |

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EXPLANATION OF PLATES

PLATE I

FIGS. 1-6. *Utricularia gibba*. Silhouettes of trap to show door posture. 1, Living trap in the set condition; 2, same after discharge; 3, same after puncturing the wall; 4, same, a portion of the cheek cut away to show the relations of the door more clearly; 5, sagittal section, living; 6, the same, fixed with iodine and cleared with glycerol. See Fig. 15-A.

FIGS. 7-10. *U. intermedia*, silhouettes of trap to show the door posture. 7, in set condition; 8, after discharge; 9, after puncturing; 10, sagittal section, living. See Fig. 16.

PLATE II

FIGS. 11-13. *U. intermedia*. Front views of the door in the same trap to show the intercellular air before discharge (Fig. 11), after discharge (Fig. 12) and when the door edge is held up by a small paper wedge. Note that the pattern formed by the air remains unchanged.

FIG. 14. *U. intermedia*. Position taken by the door when freed by cutting away just in front of the threshold and lying in water.

FIG. 15. Position taken by a narrow strip of the median part of the door when freed by cutting and lying in water.

FIG. 16. Same after plasmolysis in 0.5N potassium nitrate.

FIG. 17. *U. intermedia*. Trap before discharge, after all the trichomes on the door have been killed with iodine; and

FIG. 18. same after discharge.

FIG. 19. Front of door of *U. gibba* to show the velum lying against the surface of the door just above the edge. Note that the top edge of the velum (indicated by the pointer) is caught under the short trichomes arising from the surface of the door just below the level of the lower pair of bristles. Living.

FIG. 20. Median section through the threshold of *U. intermedia*, showing the outer (to the left of pointers), middle (between pointers) and inner zone (right of pointers) of the pavement epithelium; and showing also the velum attached to the cells of the outer zone. Remains of the cuticle walls project from between the cells of the inner zone.

FIG. 21. *U. intermedia*. Door exposing the inner surface showing the character of the epidermal cells of the inner course. See Fig. 17-A for guidance in recognising the various areas.

FIG. 22. *U. vulgaris*. Threshold flattened out to show the various zones of the pavement epithelium, the middle zone, between the paired white pointers, ending at the black pointer. The outer zone above, inner zone below, separated by single white pointer at the left. Compare with Plate V-47.

PLATE III

FIGS. 23-30. A series of sections of the threshold at different points, the positions of which can be determined by looking at Fig. 13-A (p. 397). Fig. 23, section at the extreme end of the threshold; the door seen crossing it; Fig. 24, a little lower (between 1 and 2, Fig. 13-A) the velum can be seen at the left; Fig. 25, at point 2; Fig. 26, between points 2 and 3 but nearer point 2; Fig. 26, about half way between points 2 and 3; Fig. 27, at point 3; Fig. 28, between points 3 and 4, but nearer 3; Figs. 29 and 30, at or near the middle point. This series is to show the change in form of the threshold from point to point.

FIG. 31. *U. vulgaris*. A very young, scarcely functional trap which shows well the structure of the velum. The cuticular caps of the capital cells of the middle zone form a membrane attached to the bladdery cuticles formed by the capital cells of the outer zone. Inner zone not shown.

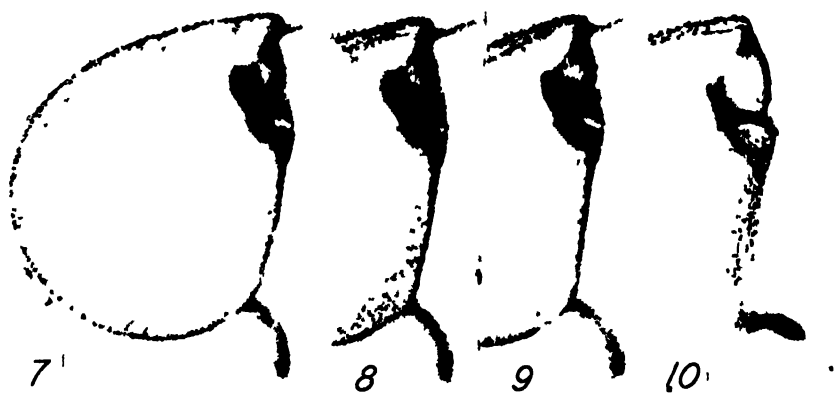
FIG. 32. Part of inner surface of door between a, b, and c, Fig. 17-A, the preparation having lain flattened out in water for some time. The cells are distorted and quite out of their natural condition, which can be seen in the corresponding area in Fig. 33.

FIG. 33. Note however that the ends of entire cells near the cut edge of the door are even here distended, having no normal living cells to oppose them. These "spring"-like cell walls are not to be found in the uninjured door. Compare Fig. 22. The completely normal form of these cells may be seen in Plate IV-35, except, again, at the cut margins, well shown at the upper left, *U. intermedia*.

FIG. 34. The cell of the inner course of the door along a narrow zone (the edge zone) showing the numerous rods. These cells are very like those of the outer hinge.

PLATE IV

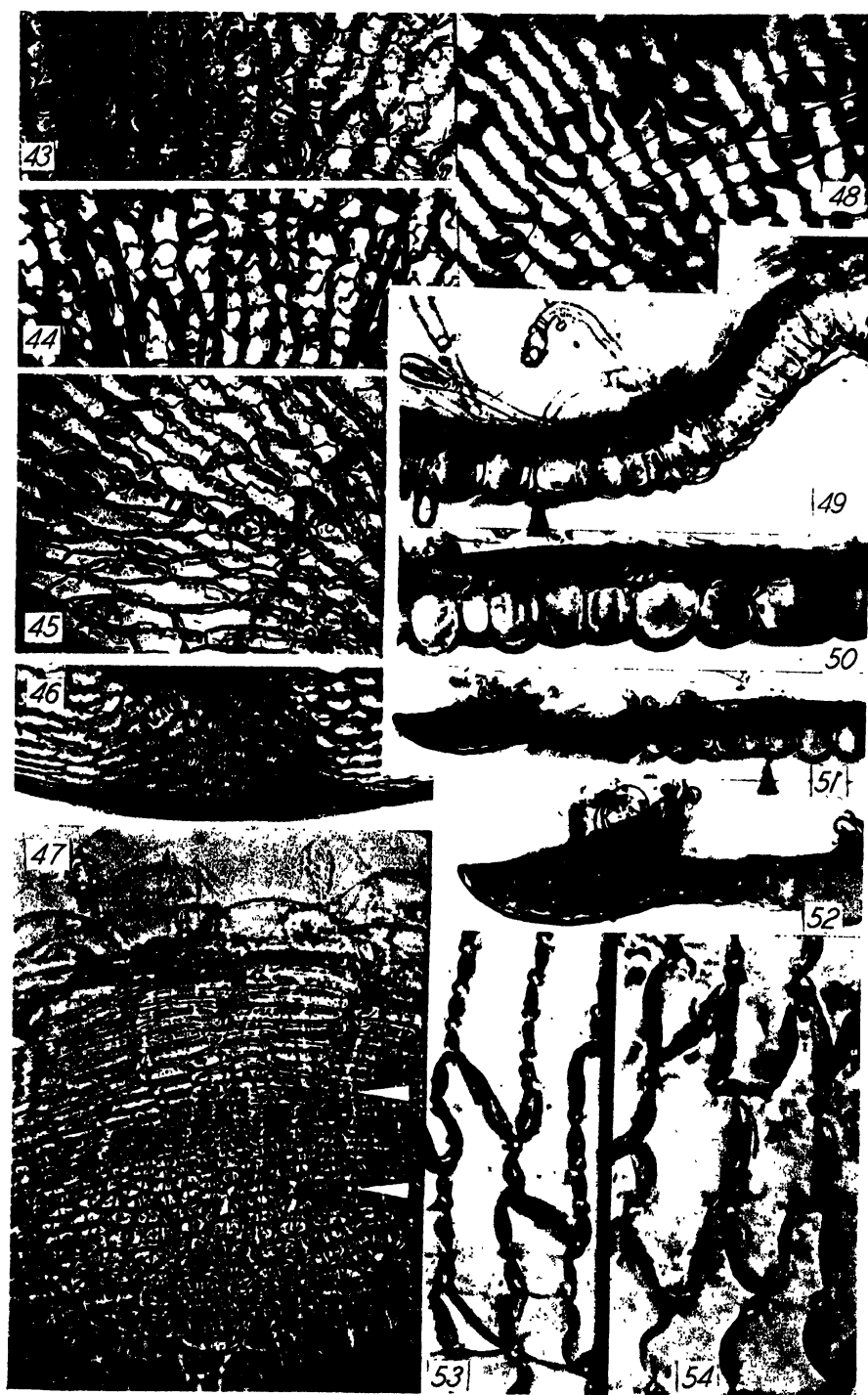
FIG. 35. Inner epidermis of the door of *U. vulgaris*. For photographic purposes advantage was taken of the presence of anthocyanin in the cells to get a "negative" picture in which the cell walls are for the most part dark. The upper hinge shown is fragmentary, being torn in dissection, but these cells show in the lower left hand corner as the hinge zone merges into the edge zone, and in Fig. 37. Note that the "circular lines" are not seen except where the middle zone cells are tilted toward the eye, and are elsewhere replaced by grooves, the infolds of the outer walls (cf. Fig. 39 of this plate). The bulbous swellings of the middle piece (indicated by the pointer) lie where the two paired groups of larger cells are seen between the central hinge and the lower half of the middle piece. The small cells of the central hinge have relatively very large rods. The details of this region are better seen in Fig. 36.











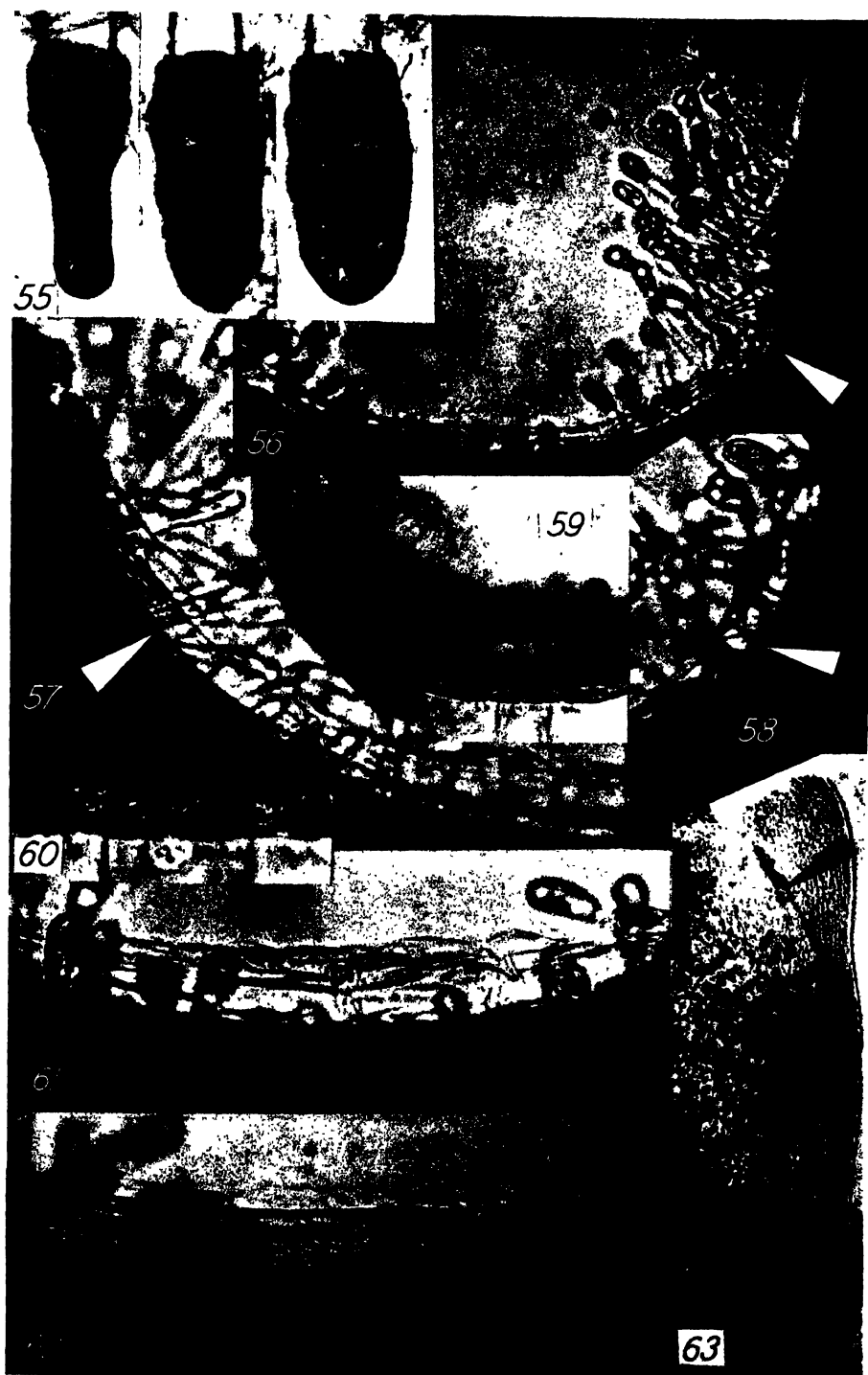


FIG. 36. The zig-zag infolds of the outer cell walls can be seen, becoming longitudinal and roughly parallel as the central hinge merges into the middle piece. This figure is the right half (approx.) of the central hinge of another preparation. The pointers indicate corresponding cells, for orientation purposes. On the upper left of Fig. 35, distorted cells with the "spring"-shaped walls.

FIG. 37. A portion of the outer hinge zone. The walls with more numerous rods. Compare with Plate V-48, in which the circular lines can be seen, only faintly indicated in this figure (Fig. 37).

FIG. 38. Sagittal section of the living door region of *U. intermedia*. The door is not quite in the normal closed position. Some details are of course obscured by shadows cast by the threshold and the insertion of the upper hinge. Compare with Fig. 13-A, which was made with this preparation as a basis.

FIG. 39. A small portion of the inner epidermis of the middle zone of the door, to show one "circular line" and the only faint double shadows made elsewhere. For optical interpretation see Fig. 23-A.

FIG. 40. Median section of the threshold of *U. vulgaris*. Middle zone between the pointers, outer on the left, inner on the right. The slight groove affording the resting place for the middle piece of the door can be seen just in front of the ample velum.

FIG. 41. Threshold of *U. aff. cucullata* (Hoene, 3070, Brazil). This is like that of *U. purpurea*. Velum in front of slightly dished narrow threshold, with a bolster of inflated cuticular membranes of the inner zone of pavement epithelium.

FIG. 42. The same but from another preparation.

PLATE V

FIGS. 43-46. Outer epidermis of the door of *U. intermedia*.

FIG. 43. Outer hinge zone (cf. Fig. 21-B).

FIG. 44. Middle zone. The shadowy walls of the inner course of cells can be seen (cf. Fig. 21-C).

FIG. 45. From tension zone along line 11', Fig. 17-A, where there is change from transverse to radial orientation of the cells (cf. Fig. 21-A).

FIG. 46. The middle piece (cf. Figs. 23-B and 24-B).

FIG. 47. En face view of the middle portion of the threshold of *U. vulgaris*, in which the character of the capital cells of the outer, middle and inner threshold zone can be seen, together with the velum attached to the outer zone cells. Only the outer cells, the capitals, of the three-celled glandular trichomes can be seen.

FIG. 48. Inner epidermis of outer hinge of door, to show the "circular lines". For optical explanation see Figs. 23-A and 24-A.

FIGS. 49-52. Sagittal section of the door, *U. intermedia*.

FIG. 49. Upper hinge zone, ending at the pointer.

FIG. 50. Middle zone; the thin inner infolded walls of the outer course can best be seen in this section.

FIG. 51. Middle zone merging into the central hinge and this into the middle piece. The infold of an outer wall of the inner epidermis, without support of rods, can be seen clearly at the pointer.

FIG. 52. The middle piece, showing the rods to be broad at the outer walls and smaller at their inner ends.

FIG. 53. Inner door epidermis, outer hinge zone, to show the numerous regularly cylindrical rods. Stained with Ruthenium red.

FIG. 54. Inner door epidermis, middle zone, showing the irregularly, more widely spaced rods, and no transverse "bars". Ruthenium red.

PLATE VI

FIG. 55. A living trap of *U. intermedia*, before and after discharge (resp. left hand and middle) and after the velum had been cut on one side (right). The result of this operation is seen in the next figure.

FIG. 56. The velum of the trap seen in Fig. 55, showing the cut, indicated by the pointer. Compare with the uninjured velum on the other side seen in

FIG. 57. Uninjured velum, on the right side, facing the door.

FIG. 58. Another experiment. Only the cut velum is shown.

FIG. 59. A door, living, removed and viewed edgewise to show the thickenings on each side of the middle point (between the pointers). Seen enlarged in

FIG. 60. Front of door facing downwards. The two thickenings of the middle piece, projecting inwardly.

FIG. 61. View of the middle reach of the velum of *U. intermedia* from the front. The membranes are derived from the bladdery cuticles of the outer zone of the threshold.

FIG. 62. The same as seen from the inside of the trap. The membrane here seen is derived from the cuticles of the middle zone of the threshold.

FIG. 63. Part of the door of a trap killed by some hours' immersion in Ruthenium red. The stain collected in the chink where the velum edge lay and consequently traced the line of the velum edge across the door. The pointer indicates the line. *U. vulgaris*.

A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.

II. THE DISTRIBUTION BY AGES IN THE EARLY STAGES OF POPULATION GROWTH¹

BY JOHN STANLEY²

Abstract

In a previous paper (2) a mathematical theory of the growth of populations of the flour beetle, *T. confusum* Duv., was developed, and a function $\theta(T)$ descriptive of the rate at which eggs are arriving at hatching age at a time, T , was described in a cursory manner. This function has now been written $\theta(T, \gamma)$ giving the number of eggs of age γ at time T , where $t_0 = 0 < T < t_3$, t_3 being the time at which the first egg hatches.

$\theta(T, \gamma)$ has been developed, and its characteristics have been described by means of a study of its partial derivatives, whence it has been possible to plot a frequency-distribution surface for the numbers of eggs of various ages during this early stage of population growth.

Introduction

In a previous paper (2), the author developed a mathematical theory descriptive of the growth of populations of the flour beetle, *T. confusum*, Duv., the populations being grown in small environments each consisting of 32 gm. of whole wheat flour.

The adequate notation set up in that paper will be used unchanged in this present investigation, and need not be repeated in tabular form.

During the discussion of the appearance of larvae in the populations (2), a function, $\theta(T)$, descriptive of the rate at which eggs are arriving at hatching age at a time T was introduced. Its form, however, was not developed.

It is now proposed to develop an extended form of $\theta(T)$ such that, at any time $t_2 = t_0 = 0 \leq T \leq t_3$, i.e., from the commencement of the growth of the population to the moment of hatching of the first egg, it shall be possible to determine the number of eggs of ages from γ to $\gamma + d\gamma$ present in the flour mass.

Discussion

It was shown, (2, p. 657) that

$$\theta(t_2) = R_2 N_{11} - \int_{t_2}^{t_3} \frac{\theta(t) C'_{11,2}}{N_2} dt, \quad (1)$$

where $C'_{11,2} = \frac{HN_2}{cN_2 + d}$, N_2 equals the number of eggs at a time T .

We shall now replace $\theta(t_2)$ by $\theta(T, \gamma)$ where T is any instant of time $t_2 \leq T \leq t_3$, and γ is the age of any egg. Then $\theta(T, \gamma)$ defines a family of frequency distributions in γ , T being thought of as a parameter determining which curve of the family shall be considered at any time.

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Then where $\alpha_1 = R\epsilon N_{11}$ (see 2, p. 649)

$$\theta(T, \gamma) = \alpha_1 - \int_{T-\gamma}^T \frac{H\theta(t, \gamma)}{cN_2 + d} dt \quad (2)$$

Equation 2 is an example of *Volterra's Equation* (1, p. 13) in the case of which, if certain necessary and sufficient conditions are satisfied, one and only one solution can be obtained, and that by the term-by-term integration of an absolutely and uniformly convergent series developed by the method of "successive substitutions."

As Equation 2 can be shown to satisfy these conditions, we proceed to its solution by the above-mentioned method.

Substituting in Equation 2 the value of $\theta(T, \gamma)$ as determined from the equation itself,

$$\theta(T, \gamma) = \alpha_1 - H \int_{T-\gamma}^T \frac{\alpha_1 - \int_{T-\gamma}^t \frac{H\theta(t, \gamma)}{cN_2 + d} dt}{cN_2 + d} dt, \quad (3)$$

from which

$$\theta(T, \gamma) = \alpha_1 - H\alpha_1 \int_{T-\gamma}^T \frac{dt}{cN_2 + d} + H^2 \int_{T-\gamma}^T \frac{dt}{cN_2 + d} \int_{T-\gamma}^t \frac{\theta(t, \gamma)}{cN_2 + d} dt. \quad (4)$$

By successive repetitions of this process there is obtained the absolutely and uniformly convergent series:

$$\begin{aligned} \theta(T, \gamma) = & \alpha_1 - H\alpha_1 \int_{T-\gamma}^T \frac{dt}{cN_2 + d} + H^2\alpha_1 \int_{T-\gamma}^T \frac{dt}{cN_2 + d} \int_{T-\gamma}^t \frac{dt}{cN_2 + d} \\ & - H^3\alpha_1 \int_{T-\gamma}^T \frac{dt}{cN_2 + d} \int_{T-\gamma}^t \frac{dt}{cN_2 + d} \int_{T-\gamma}^t \frac{dt}{cN_2 + d} + \dots \text{etc.} \end{aligned} \quad (5)$$

It was shown (2, Eq. 27) that

$$\frac{dN_2}{dt} = \frac{aN_2 + b}{cN_2 + d}, \quad (6)$$

which, as $a < 0$ (2, p. 648), may be written

$$\frac{dN_2}{dt} = \frac{b - |a|N_2}{cN_2 + d}. \quad (7)$$

From Equation 7,

$$dt = \frac{(cN_2 + d)}{(b - |a|N_2)} dN_2. \quad (8)$$

Substituting the value of dt from Equation 7 in Equation 5, and denoting by $F(T)$ the value of N_2 at time T ,

$$\theta(T, \gamma) = \alpha_1 - H\alpha_1 \int_{F(T-\gamma)}^{F(T)} \frac{dN_2}{b - |a|N_2} + H^2\alpha_1 \int_{F(T-\gamma)}^{F(T)} \frac{dN_2}{b - |a|N_2} \int_{F(T-\gamma)}^{F(t) = N_2} \frac{dN_2}{b - |a|N_2} - \dots \text{etc.} \quad (9)$$

Integrating term by term, as it is legitimate to do, by reason of the fact that Equation 2 satisfied the required necessary and sufficient conditions, and writing

$$\Phi = -\frac{H}{|a|} \left[\log_e \left\{ b - |a| F(T) \right\} - \log_e \left\{ b - |a| F(T-\gamma) \right\} \right], \quad (10)$$

it is found that

$$\theta(T, \gamma) = c_1 + \alpha_1 \left[1 - \Phi + \frac{\Phi^2}{2!} - \frac{\Phi^3}{3!} + \frac{\Phi^4}{4!} - \dots \text{etc.}, \quad (11)$$

where c_1 is the sum of all the constants of integration.*

The series in Φ converges to the value $e^{-\Phi}$ for all values of Φ , $-\infty < \Phi < +\infty$, hence

$$\theta(T, \gamma) = c_1 + \alpha_1 e^{-\Phi} = c_1 + \alpha_1 \left[\frac{b - |a| F(T-\gamma)}{b - |a| F(T)} \right]^{-\frac{H}{|a|}} \quad (12)$$

When $T=0$, $\gamma=0$, as no eggs were laid prior to $T=t_2=0$. Also, when $T=0$, $N_2 = F(0) = 0$, and $\theta(T, 0) = R_e N_{11}$ (see 2, p. 649).

Hence

$$\theta(T, 0) = R_e N_{11} = c_1 + \alpha_1 \left(\frac{b}{b} \right)^{-\frac{H}{|a|}} \quad (13)$$

so that $c_1 = 0$.

It must be remembered that $a < 0$. Hence to avoid confusion, we write

$$\theta(T, \gamma) = R_e N_{11} \left[\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right]^{\frac{H}{|a|}} \quad (14)$$

As $\theta(T, \gamma)$ determines the number of eggs of age γ at a time T , $t_2 \leq T \leq t_3$, clearly, at any time T , the sum of the eggs of all ages from $\gamma=0$ to $\gamma=T$ must be equal to $F(T)$, the total number of eggs at time T , i.e.,

$$\int_{\gamma=0}^{\gamma=T} R_e N_{11} \left[\frac{b - |a| F(t)}{b - |a| F(t-\gamma)} \right]^{\frac{H}{|a|}} dt = F(T) \quad (15)$$

We proceed to show that this is so. Set $t-\gamma=Z$, whence $-d\gamma=dZ$. When $\gamma=0$, $T-\gamma=T=Z$; when $\gamma=T$, $T-\gamma=0=Z$, and the integral becomes, reversing the limits to eliminate the minus sign of dZ ,

$$R_e N_{11} \left[b - |a| F(T) \right] \int_0^T \frac{dZ}{[b - |a| F(Z)]^{\frac{H}{|a|}}} \quad (16)$$

But $F(Z) = N_2$ at the point Z , whence, setting $R_e N_{11} \left[b - |a| F(T) \right]^{\frac{H}{|a|}} = \beta$,

we obtain

$$\beta \int_0^T \frac{dZ}{[b - |a| N_2]^{\frac{H}{|a|}}} \quad (17)$$

*That this sum is finite follows from the absolute and uniform convergence of the series of Equation 9.

From (2, Eq. 27), $dZ = \frac{(cN_2+d)}{(b-|a|N_2)} dN_2$.

When $Z=0$, $N_2=0$, and when $Z=T$, $N_2=F(T)$ whence the integral becomes

$$\beta \int_0^{F(T)} \frac{(cN_2+d)}{(b-|a|N_2)^{\frac{H}{|a|}+1}} dN_2. \quad (18)$$

Set $b-|a|N_2=y$, then $cN_2+d = \frac{cy-(bc+|a|d)}{-|a|}$, and $dN_2 = \frac{y}{-|a|}$.

When $N_2=0$, $y=b$, and when $N_2=F(T)$, $y=b-|a|F(T)$, so that the integral becomes

$$\frac{\beta}{(-|a|)^2} \int_b^{b-|a|F(T)} \left[cy - \frac{H}{|a|} - (bc+|a|d)y \left(-\frac{H}{|a|} - 1 \right) \right] dy. \quad (19)$$

This is immediately integrable, and becomes

$$\frac{R\epsilon N_{11}}{(-|a|)^2} \left[b-|a|F(T) \right] \frac{H}{|a|} \left[\frac{-|a|cy}{H-|a|} \left(-\frac{H}{|a|} + 1 \right) + \frac{(bc+|a|d)(|a|y)^{-\frac{H}{|a|}}}{H} \right]_b^{b-|a|F(T)}. \quad (20)$$

From (2, p. 648), $H-|a|=R\epsilon N_{11}c$, whence on substituting the limits and simplifying somewhat, we obtain

$$-\frac{[b-|a|F(T)]}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)} + \frac{R\epsilon N_{11}[b-|a|F(T)]}{|a|} \frac{H}{|a|} \left[\frac{b \left(-\frac{H}{|a|} + 1 \right)}{R\epsilon N_{11}} - \frac{(bc+|a|d)b^{-\frac{H}{|a|}}}{H} \right]. \quad (21)$$

Consider the two terms within the square brackets on the right. From (2, p. 648), $b=R\epsilon N_{11}d$, whence

$$\frac{b}{H} \left(-\frac{H}{|a|} + 1 \right) = \frac{b \left(-\frac{H}{|a|} + 1 \right)}{HR\epsilon N_{11}d},$$

so that the last term of (21) is

$$\begin{aligned} & \frac{[b-|a|F(T)]}{|a|} \frac{H}{|a|} \left[\frac{Hdb \left(-\frac{H}{|a|} + 1 \right) - (bc+|a|d)b \left(-\frac{H}{|a|} + 1 \right)}{Hd} \right] \\ &= \frac{[b-|a|F(T)]}{|a|} \frac{H}{|a|} \cdot \frac{b \left(-\frac{H}{|a|} + 1 \right) (Hd-bc-|a|d)}{Hd}. \end{aligned} \quad (22)$$

But, from (2, p. 648) $Hd-bc-|a|d=|a|d+R\epsilon N_{11}cd-|a|d-R\epsilon N_{11}cd=0$, whence the last term on the right of formula (21) is zero, and there remains only

$$-\frac{[b-|a|F(T)]}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)},$$

which, from (2, p. 648),

$$\begin{aligned} &= F(T) - \frac{b}{|a|} + \frac{R\epsilon N_{11}(bc+|a|d)}{H(|a|)} = F(T) - \frac{1}{H(|a|)} \left[bH - R\epsilon N_{11}(bc+|a|d) \right] \\ &= F(T) - \frac{1}{H(|a|)} \left[b(|a|+R\epsilon N_{11}c) - R\epsilon N_{11}bc - R\epsilon N_{11}(|a|d) \right] \end{aligned} \quad (23)$$

$$= F(T) - \frac{1}{H(|a|)} \left[(|a|)b - R_e N_{11} |a| d \right] = F(T) \quad (\text{Q.E.D.}) \quad (24)$$

Characteristics of the Frequency-distribution Surface Defined by $\theta(T, \gamma)$

As the operations of finding the various derivatives of $\theta(T, \gamma)$ involve only the ordinary rules of partial differentiation, the intermediate steps may be omitted. It is thus seen that:

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \theta(T, \gamma) = R_e N_{11} > 0, \quad (25)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T-\gamma) \rightarrow T} \theta(T, \gamma) = R_e N_{11} > 0, \quad (26)$$

$$\lim_{T \rightarrow \infty}^* = \lim_{F(T) \rightarrow \xi} \theta(T, \gamma) = 0, \quad (27)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T-\gamma) \rightarrow 0} \theta(T, \gamma) = R_e N_{11} \left[1 - \frac{|a|}{b} F(T) \right]^{\frac{H}{|a|}} > 0. \quad (28)$$

$$\frac{\partial \theta(T, \gamma)}{\partial T} = -R_e N_{11} H \left[\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right]^{\frac{H}{|a|}} \left[\frac{1}{c F(T) + d} - \frac{1}{c F(T-\gamma) + d} \right] > 0, \quad (29)$$

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \frac{\partial \theta}{\partial T} = 0, \quad (30)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T-\gamma) \rightarrow T} \frac{\partial \theta}{\partial t} = 0, \quad (31)$$

and, as from (2, p. 648) $\xi = \frac{b}{|a|}$,

$$\lim_{T \rightarrow \infty} = \lim_{F(T) \rightarrow \xi} \frac{\partial \theta}{\partial T} = 0, \quad (32)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T-\gamma) \rightarrow 0} \frac{\partial \theta}{\partial T} = -R_e N_{11} H \left[1 - \frac{|a|}{b} F(T) \right] \left[\frac{1}{c F(T) + d} - \frac{1}{d} \right] > 0. \quad (33)$$

$$\frac{\partial \theta(T, \gamma)}{\partial \gamma} = -\frac{R_e N_{11} H}{[c F(T-\gamma) + d]} \left[\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right]^{\frac{H}{|a|}} < 0, \quad (34)$$

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \frac{\partial \theta}{\partial \gamma} = -\frac{R_e N_{11} H}{d} < 0, \quad (35)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T-\gamma) \rightarrow T} \frac{\partial \theta}{\partial \gamma} = -\frac{R_e N_{11} H}{c F(T) + d} < 0, \quad (36)$$

$$\lim_{T \rightarrow \infty} = \lim_{F(T) \rightarrow \xi} \frac{\partial \theta}{\partial \gamma} = 0, \quad (37)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T-\gamma) \rightarrow 0} \frac{\partial \theta}{\partial \gamma} = -\frac{R_e N_{11} H}{c F(T) + d} \left[1 - \frac{|a|}{b} F(T) \right]^{\frac{H}{|a|}} < 0. \quad (38)$$

* In an actual case, these formulations hold only if $T < b$, but it is of interest to suppose hatching to be in some way indefinitely deferred, and to examine the consequences.

$$\frac{\partial^2 \theta(T, \gamma)}{\partial T \partial \gamma} = \frac{\partial^2 \theta(T, \gamma)}{\partial \gamma \partial T} = \frac{R_e N_{11} H}{[cF(T-\gamma) + d]} \left[\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right] \cdot \left[\frac{H}{|a|} \left\{ \frac{c \{ b - |a| F(T-\gamma) \}}{\{ cF(T-\gamma) + d \}^2} - H \left\{ \frac{1}{cF(T) + d} - \frac{1}{cF(T-\gamma) + d} \right\} \right\} \right] > 0. \quad (39)$$

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \frac{\partial^2 \theta}{\partial T \partial \gamma} = \frac{R_e N_{11} H b c}{d^3} = \frac{(R_e N_{11})^2 H c}{d^3} > 0, \quad (40)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T-\gamma) \rightarrow T} \frac{\partial^2 \theta}{\partial T \partial \gamma} = \frac{R_e N_{11} H c [b - |a| F(T)]}{[cF(T) + d]^3} > 0, \quad (41)$$

$$\lim_{T \rightarrow \infty} = \lim_{F(T) \rightarrow \xi} \frac{\partial^2 \theta}{\partial T \partial \gamma} = 0, \quad (42)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T-\gamma) \rightarrow 0} \frac{\partial^2 \theta}{\partial T \partial \gamma} = \frac{R_e N_{11} H [b c - H(d^2 - d)]}{d^3 [cF(T) + d]} \left[1 - \frac{|a|}{b} F(T) \right] \cdot \frac{H}{|a|} > 0. \quad (42a)$$

$$\frac{\partial^2 \theta(T, \gamma)}{\partial T^2} = -R_e N_{11} H \left(\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right) \cdot \frac{H}{|a|} \left[\frac{1}{cF(T) + d} - \frac{1}{cF(T-\gamma) + d} \right] + R_e N_{11} H \left(\frac{b - |a| F(T)}{b - |a| F(T-\gamma)} \right) \cdot \frac{H}{|a|} \left[\frac{c \left\{ \frac{b - |a| F(T-\gamma)}{cF(T-\gamma) + d} \right\}}{\{ cF(T-\gamma) + d \}^2} - \frac{c \left\{ \frac{b - |a| F(T)}{cF(T) + d} \right\}}{\{ cF(T) + d \}^2} \right] < 0 \quad (43)$$

That $\frac{\partial^2 \theta(T, \gamma)}{\partial T^2} < 0$ may be shown as follows:

Let $cF(T) + d = x$, $cF(T-\gamma) = y$, then $x > y$, and the discriminant, D , of $\frac{\partial^2 \theta(T, \gamma)}{\partial T^2}$ is the quantity within the square brackets, i.e.,

$$D = -H \left(\frac{1}{x} - \frac{1}{y} \right)^2 + \frac{cF'(T-\gamma)}{y^3} - \frac{cF'(T)}{x^3} \quad (44)$$

$$D = -\frac{H}{x^2} + \frac{2H}{xy} - \frac{H}{y^2} + \frac{cF'(T-\gamma)}{y^3} - \frac{cF'(T)}{x^3}$$

As $x > y$, $x^2 > y^2$; also $F'(T-\gamma) > F'(T)$ from (2, Eq. 30), where $0 < F(T) < \xi$

$$\text{Whence } \frac{cF'(T-\gamma)}{y^3} - \frac{cF'(T)}{x^3} > 0 \quad (46)$$

$$\text{also } \frac{H}{xy} - \frac{H}{x^2} > 0, \quad \frac{H}{xy} - \frac{H}{y^2} < 0, \quad (47)$$

$$\text{whence } \frac{cF'(T-\gamma)}{y^3} - \frac{cF'(T)}{x^3} + \frac{H}{xy} - \frac{H}{x^2} > 0. \quad (48)$$

Now $-\frac{H}{x^2} > -\frac{H}{y^2}$, whence, from Equation 40,

$$\frac{cF'(T-\gamma)}{y^3} - \frac{cF'(T)}{x^3} - \frac{H}{x^2} > -\frac{H}{x^2} > -\frac{H}{y^2}, \quad (49)$$

*By $F'(T-\gamma)$ is meant the first partial derivative $\frac{\partial F(T-\gamma)}{\partial T}$

and as obviously $\frac{H}{xy} = \frac{H}{xy}$, it is seen that $D > 0$, whence $\frac{\partial^2 \theta(T, \gamma)}{\partial T^2} < 0$.

Further, it can be shown that:

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \frac{\partial^2 \theta(T, \gamma)}{\partial T^2} = 0, \quad (50)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T - \gamma) \rightarrow T} \frac{\partial^2 \theta}{\partial T^2} = 0, \quad (51)$$

$$\lim_{T \rightarrow \infty} = \lim_{F(T) \rightarrow \xi} \frac{\partial^2 \theta}{\partial T^2} = 0, \quad (52)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T - \gamma) \rightarrow 0} \frac{\partial^2 \theta}{\partial T^2} = 0. \quad (53)$$

$$\frac{\partial^2 \theta(T, \gamma)}{\partial \gamma^2} = R \epsilon N_{11} II \left[\frac{b - |a| F(T)}{b - |a| F(T - \gamma)} \right] \frac{H}{|a|} \left[H \left\{ \frac{1}{c F(T - \gamma) + d} \right\}^2 + \frac{c \left\{ b - |a| F(T - \gamma) \right\}}{[c F(T - \gamma) + d]^3} \right] > 0, \quad (54)$$

$$\lim_{T \rightarrow 0} = \lim_{F(T) \rightarrow 0} \frac{\partial^2 \theta}{\partial \gamma^2} = R \epsilon N_{11} II \left(\frac{Hd + bc}{d^3} \right) > 0, \quad (55)$$

$$\lim_{\gamma \rightarrow 0} = \lim_{(T - \gamma) \rightarrow T} \frac{\partial^2 \theta}{\partial \gamma^2} = \frac{R \epsilon N_{11} II [H + c F'(T)]}{[c F(T) + d]^3} > 0, \quad (56)$$

$$\lim_{T \rightarrow \infty} = \lim_{F(T) \rightarrow \xi} \frac{\partial^2 \theta}{\partial \gamma^2} = 0, \quad (57)$$

$$\lim_{\gamma \rightarrow T} = \lim_{(T - \gamma) \rightarrow 0} \frac{\partial^2 \theta}{\partial \gamma^2} = \frac{R \epsilon N_{11} II (Hd + bc)}{d^3} \left[1 - \frac{|a|}{b} F(T) \right] > 0. \quad (58)$$

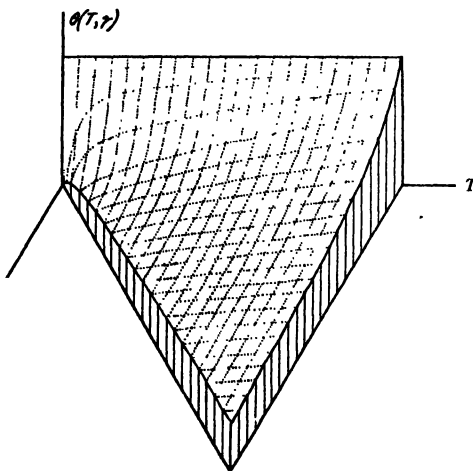


FIG. 1 General form of the frequency-distribution surface $\theta(T, \gamma)$.

From the information obtained in Equations (25) to (58) it is possible to plot the surface $\theta(T, \gamma)$ in character as shown in Fig. 1.

Thus, under the conditions set forth, the number of eggs of age γ at a time T is a function of T and γ , Equation (14). Moreover, as T increases from 0 to $+\infty$, the number of eggs of any age γ , ($0 < \gamma < \infty$) increases (Equation 29) at an ever decreasing rate (Equation 43). The number of eggs of age zero, *i.e.*, those just laid, remains however at $R \epsilon N_{11}$ (Equation 26), while the number of the oldest eggs, *i.e.*, those of age T , increases according to Equation (28),

Also, at any time the older eggs are less numerous than the younger, (Equation 34), though this difference, for equal differences in age, becomes less and less apparent as T increases (Equation 39).

Moreover, as N_2 approaches ξ , (at an infinite time,) the numbers of eggs of infinite age approaches zero, these having been entirely eliminated by prolonged exposure to eating (Equation 28)*.

It is of interest to examine $\frac{H}{|a|}$

It can be shown from (2, p. 648) that

$$\frac{H}{|a|} = \frac{E_{11}}{E_{11} - R_e W_2 A_2}$$

and as E_{11} is the maintenance rate of ingestion of a mature adult (2, p. 647) and $R_e W_2 A_2$ the rate of expenditure of assimilable nutrient material in the form of eggs, $\frac{H}{|a|}$ is the reciprocal of the "efficiency" of a mature adult as a growing locomotory machine.

The biological substantiation of the above described formulations would of course necessitate the determination of the ages of numerous eggs. This may be accomplished by determining the additional time (at the same temperature as that at which the original experiment was performed) necessary for the hatching of the particular eggs under consideration. The probable errors, (see 2, Table I) are less than .1 day for temperatures between 27° and 32° C., and only .7 day at temperatures as low as 17° C.

The writer hopes to carry out this biological substantiation in the near future.

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*This may be seen by placing $F(T) = N_2 = \xi = \frac{b}{|a|}$ in Equation 28, when the term in the square brackets becomes zero.

DURATION OF META-STABLE STATES, NEON¹

By J. M. ANDERSON

Abstract

The rate of decay of absorption of six neon lines λ 6402, λ 6334, λ 6266, λ 6163, λ 6143 and λ 5945 has been measured for a series of pressures at room temperature and at the temperature of liquid air. Curves showing the variation of half-life with pressure have been plotted for each line at both temperatures. It is found

that the function $\log \frac{I}{I_0} = \frac{D_1 - D_2}{\gamma}$ is exponential in the time, and that the rate of decay is markedly different at the same pressure and temperature for different lines ending in the same meta-stable state.

In previous papers the author has dealt with a simple theory of meta-stable atomic states, especially those of the inert gases (1) and has given experimental determination of the duration of the absorption shown by λ 7635 in argon, $^3P_2(p^6p) - ^3P_2(p^6s)$, of which the lower state is meta-stable (2). This work has now been extended to the neon spectrum and the decay curves for the absorption as shown by several lines have been determined for a series of pressures both at room temperature and at the temperature of liquid air.

Apparatus

The apparatus used is the same as that described in the previous paper (2) with only such changes as were necessitated by repair work. The electrical circuit is shown in (2, Fig. 4). G_1 and G_2 are two 750 watt two-pole 50 cycle A.C. generators using 220 volts D.C. input and giving 110 volts A.C. output. These are coupled on the same shaft by the variable coupling C which is graduated in four hundred equal divisions.

The output of G_1 excites transformer T_1 whose output passes through the rectifying valves V , the resistance CR_1 , the discharge tubes D_1 and D_2 and the synchronous switch B_1 which is fastened to the shaft of the exciting generator. The output of T_1 is shunted through the condenser C_1 which takes up the surges caused by the breaking of the circuit by B_1 . The essential feature of this circuit is that the switch B_1 must make a positive metal-to-metal contact in order to assure sufficiently low excitation to allow the lines emitted to be absorbed by an undisturbed gas. Flashes of excitation in the tube D_2 are found to last for about 10^{-4} sec. and are not followed by stray discharge due to the action of the switch B . This switch also serves to assure that the flash will occur at precisely the same time in each cycle.

The output of G_2 excites transformer T_2 whose output passes through the resistance of CR_2 , the meter $M.A.$, the discharge tubes D_3 and D_4 and the synchronous switch B_2 . A condenser is also shunted across this transformer. The essential feature is that the switch B_2 depends on the breaking down of an air gap to complete the circuit. This renders the excitation in tube D_4 rather high, but ensures flashes of an extremely short duration ($<10^{-5}$ sec.). High

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Contribution from the Physical Laboratory, University of Toronto, Toronto, Canada.

excitation is fortunately not a matter of concern in this circuit as the absorption by D_1 takes place after the discharge has been cut off so that the atoms are in zero field. Only one-half the cycle is used from both T_1 and T_2 in order to allow a longer time between successive excitations of the tubes.

The experimental method consists in producing excited atoms in tube D_1 by means of a discharge, and observing the amount of absorption suffered by various lines in the flash of light emitted by D_2 a known time after the discharge in D_1 has ceased. This known time is varied by means of the coupling C fixing the relative phase of the armatures and switches simultaneously. The tubes D_1 and D_2 are observed by means of their reflections in the mirror M set at an angle to the shaft of the generators. Thus, the character of the discharge given by the two circuits and the time difference between the two flashes given by them can be definitely checked.

The absorption tube used is shown in (2, Fig. 6). It is necessarily enclosed in a vacuum flask for low temperature work. Tungsten electrodes were used which were heated white hot at frequent intervals to ensure that all impurities were driven off. The emission tube consisted of a Geissler tube bent back and forth several times to increase the luminous area without increasing the depth of emitting gas. It was placed directly below the absorption tube. Light passed up through the absorption tube was reflected at the mirror placed in the metal cover, out to a high light-power spectrograph. By means of a system of mirrors, light from the same emission tube impressed a comparison spectrum on the plate at the same time as the exposure was being taken. This precaution was found to be absolutely essential to accurate estimation of the absorption.

Experimental

The neon used was partly purified by passage over charcoal immersed in liquid air. It was then admitted to a bulb in which a heavy discharge was passed between a potassium pool and an auxiliary electrode. This process produced a gas of very high purity in a very short time. The gas used in the emission tube was only roughly purified, as traces of impurity in the emission tube can have no effect on the life of the absorption shown by the gas in the absorption tube, and changes in intensity due to slight variations of purity were automatically cancelled out by the method of taking the comparison spectrum as described above. Ilford Special Rapid Panchromatic plates were used, developed in glycine. Density curves were made on the Moll self-recording microphotometer, densities being calculated from the formula $D_2 - D_1 = \log \frac{h_0 - d_1}{h_0 - d_2}$, in which D_1 and D_2 are the photographic densities produced

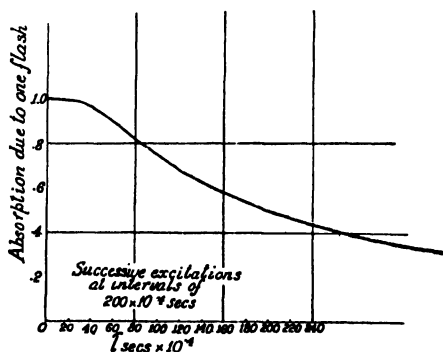


FIG. 1. Effect of successive excitations.

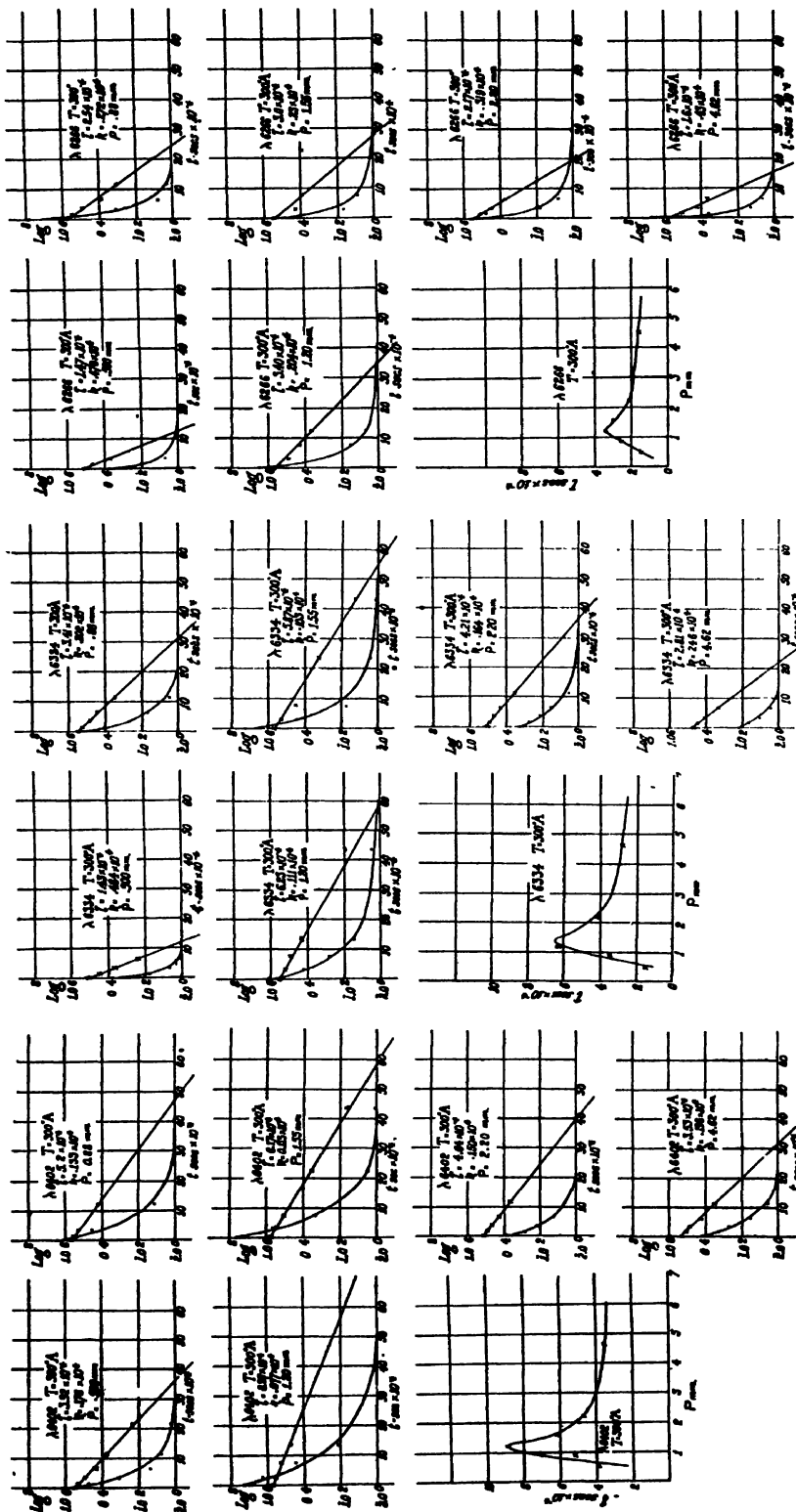


FIG. 2. Decay curves of λ 6402; $T = 300^\circ A$.

FIG. 3. Decay curves of λ 6334; $T = 300^\circ A$.

FIG. 4. Decay curves of λ 6266; $T = 300^\circ A$.

on the plate by the absorbed and comparison spectra, h_0 the total microphotometer deflection from clear plate to saturation density, and d_1 and d_2 the deflections of the microphotometer produced by the lines of the comparison and absorbed spectra. The law $\frac{D_1 - D_2}{\gamma} = \log \frac{I_1}{I_2}$ in which I_1 and I_2 are the actual intensities of light in the spectra, and γ is a constant pertaining to the plate, was assumed as the connection between I and D . This was found to be true within the limits of experimental error for the intensities and wave-lengths studied. Pressures were measured by means of a McLeod gauge.

The current through the absorption tube was kept constant by means of the meter directly in series with the tube. The current to produce the same absorption was necessarily smaller for liquid air temperatures than for room temperatures, as in these cases the excited atoms have not completely disappeared in the 1/50 sec. which elapses between successive excitations of the absorption tube. The absorption is then represented by a series:

$$A = C_e^{-kt} + C_e^{-k(t+T)} + C_e^{-k(t+2T)} = C_e^{-kt} \cdot \frac{1}{1 - e^{-kT}} = C_1 e^{-kt}$$

It will be noted that the absorption still decays exponentially with time but with a different and larger constant C . The effect on the absorption is shown in Fig. 1, in which it is seen that the effect is only appreciable when τ , the half-life, is comparable with T the period of excitation. It was found that within the limits for which absorption was experimentally observable the half-life was, as expected, independent of the exciting current. Owing to the peculiar wave form of the current (flashes of the order of 10^{-5} sec. once every $\frac{1}{50}$ sec.), D.C. calibration means little. However, the meter reading used corresponded to D.C. readings of approximately 100 micro-amperes.

Results

The results obtained are shown in the Figs. 2 to 13, in which the experimental values of $D_2 - D_1 = \log \frac{h_0 - d_1}{h_0 - d_2}$ are shown in heavy black dots (the units are arbitrary and not strictly comparable throughout). The crosses are the logarithms of these values on the scale shown. Half-life values were obtained from the slope of the straight lines through these crosses. It may be pointed out that the curves drawn through the values of $D_1 - D_2$ are intended merely to indicate the general course of the absorption and are not to be considered as giving an accurate value as, owing to the scale necessary for publication, it was found impossible to draw the steeper curves accurately. By the use of more convenient scales of plotting, half-life values as read from the absorption curves agreed with those obtained from the slope of the straight line within the error of either. It will be seen that the plotting of $\log (D_1 - D_2)$ against time gives an excellent straight line especially at the liquid air temperatures, a result fully as good as could be expected from absorption measurements which in themselves contain an error of the order of 5%. The better agreement at the low temperature is due largely to the minimizing of the effect of the error in

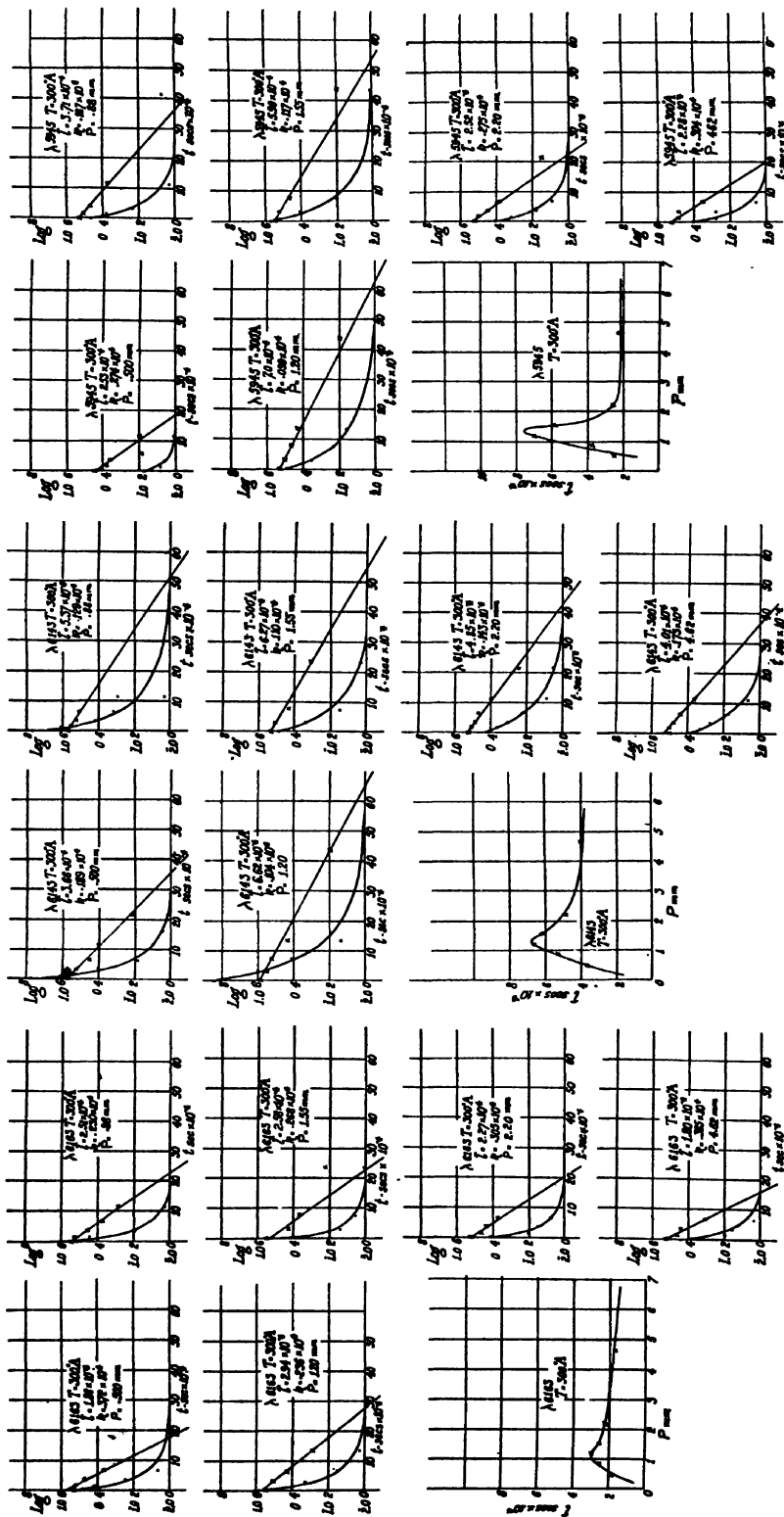


FIG. 5. Decay curves of $\lambda 6163$; $T = 300^\circ \text{A}$.

FIG. 6. Decay curves of $\lambda 6143$; $T = 300^\circ \text{A}$.

FIG. 7. Decay curves of $\lambda 5945$; $T = 300^\circ \text{A}$.

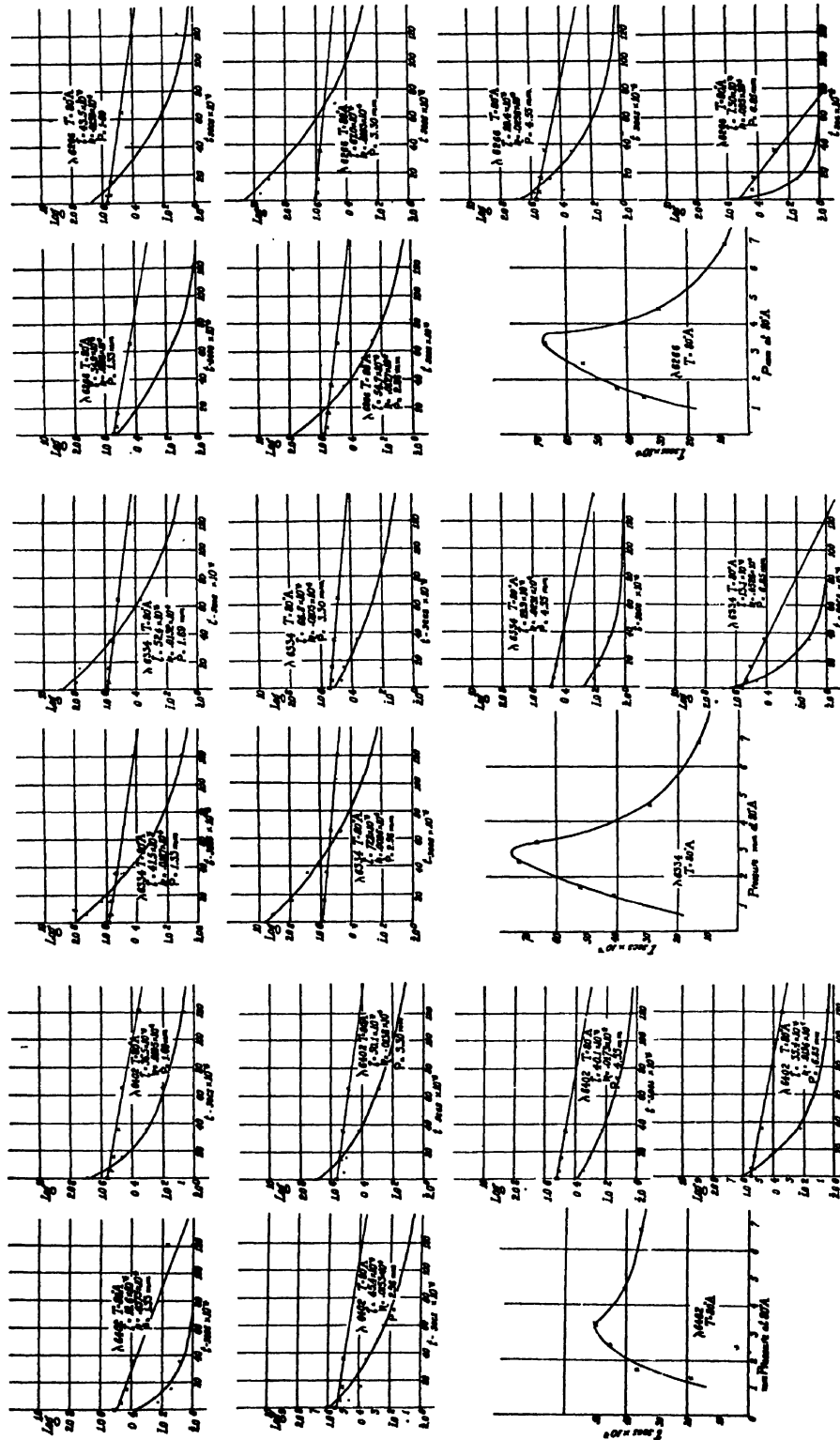


FIG. 8. Decay curves of $\lambda 6402$; $T = 80^\circ \text{ A}$.

FIG. 9. Decay curves of $\lambda 6334$; $T = 80^\circ \text{ A}$.

FIG. 10. Decay curves of $\lambda 6266$; $T = 80^\circ \text{ A}$.

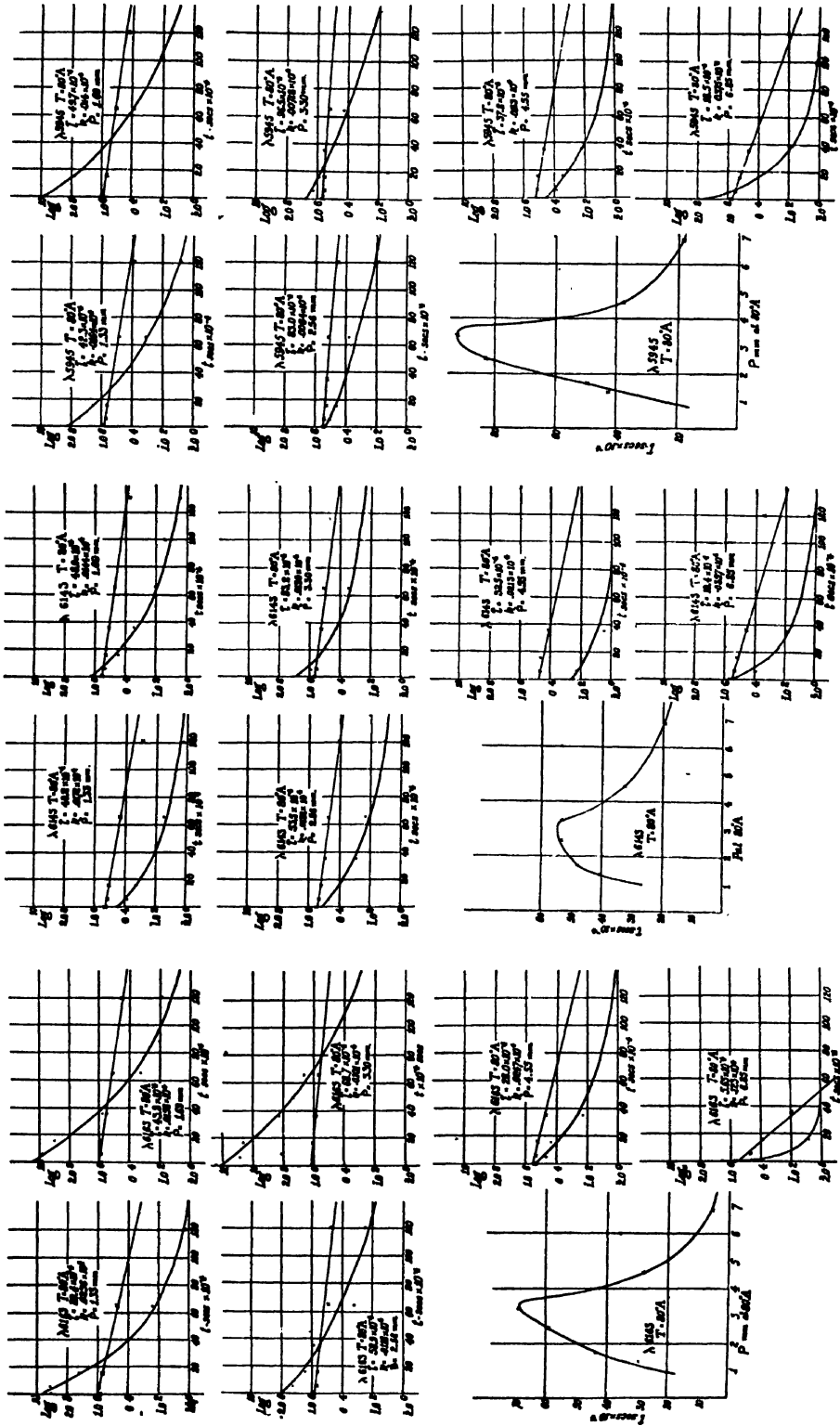


FIG. 11. Decay curves of λ 6163; $T = 80^\circ \text{ A}$.

FIG. 12. Decay curves of λ 6143; $T = 80^\circ \text{ A}$.

FIG. 13. Decay curves of λ 5945; $T = 80^\circ \text{ A}$.

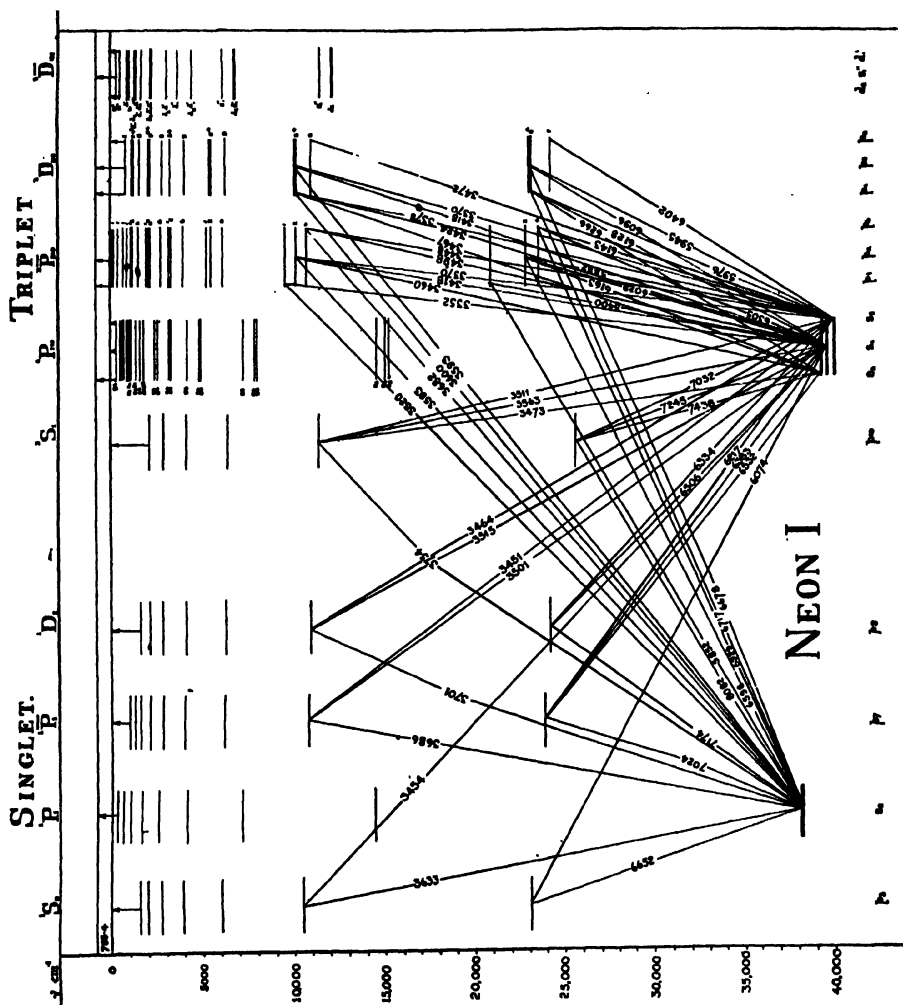


FIG. 14. Neon energy levels.

time difference in this case. The fact that $\log (D_1 - D_2)$ plotted against t is a straight line justifies us in assigning a half-life value to the function $D_1 - D_2$. The decay of $(D_1 - D_2)$ is plotted for six lines ending in the meta-stable states of neon (See Fig. 14): λ 6402, ($^3D_3 - ^3P_2$); λ 6334, ($^1D_2 - ^3P_2$); λ 6266, ($^3D_1 - ^3P_0$); λ 6163, ($^3P_1 - ^3P_0$); λ 6143, ($^3P_2 - ^3P_2$); λ 5945, ($^3D_2 - ^3P_2$). Maximum absorption of the order of 75% was obtained with these lines. For lines whose end states were 3P_1 or 1P_1 absorptions of more than 10% were never found, and absorption was only observable for very small time differences, so that no curves have been plotted for these. The time difference was set by means of the coupling C with an accuracy of at least 0.25×10^{-4} sec. Settings were not made at less than 5.0×10^{-4} sec. as for very short time differences the absorption should obey a more complicated law (1), due to the fact that the atoms are far from diffusion equilibrium. This time therefore (equals approximately 5×10^{-4} sec) is used as the arbitrary zero time of the curves shown. The variation of half-life with pressure is shown in the lower right hand section of each figure. The results upon which these are based are shown in Table I.

TABLE I
VALUES OF τ USED IN COMPUTATIONS OF VARIATION OF HALF-LIFE WITH PRESSURE

P, mm.	λ 6402	λ 6334	λ 6266	λ 6163	λ 6143	λ 5945	Number per cc.
	τ , sec. $\times 10^{-4}$						
T, 80° Absolute							
1.33	18.6	41.5	34.9	29.4	40.2	42.3	16.2×10^{16}
1.69	36.5	52.4	43.5	43.8	48.0	49.7	20.6×10^{16}
2.56	45.4	72.8	54.7	58.9	53.5	83.0	31.2×10^{16}
3.30	50.1	66.8	67.0	68.7	53.2	96.5	40.2×10^{16}
4.55	40.1	29.9	29.4	28.0	32.5	37.8	55.5×10^{16}
6.58	35.4	13.1	7.30	5.65	19.4	18.5	80.0×10^{16}
T, 300° Absolute							
.500	3.92	1.43	1.47	1.86	3.66	2.53	1.62×10^{16}
.88	5.20	3.41	2.54	2.51	5.37	3.71	2.85×10^{16}
1.20	8.97	6.23	3.40	2.94	6.62	7.00	3.89×10^{16}
1.55	6.17	5.87	3.00	2.58	6.27	5.90	5.03×10^{16}
2.20	4.64	4.21	2.17	2.27	4.85	2.52	7.13×10^{16}
4.62	3.53	2.81	1.60	1.80	4.01	2.28	15.00×10^{16}

Discussion

It will be noted that the half-life value plotted against pressure gives very different curves for different transitions even though these end in the same lower state. Not only are the absolute magnitudes different but the shape of the curve seems different for different transitions. Thus we see that at liquid

air temperatures λ 6402 and λ 5945, both ending in the 3P_2 state, differ by a factor of almost two in the height of the maximum, a difference which is far beyond the limit of error. The lines λ 6402 and λ 6143 give curves which are of a similar nature and are not so different as to preclude their being actually the same. The line λ 6334 is definitely different from these and also from the line λ 5945. At this temperature λ 6163 and λ 6266, both ending in 3P_0 , give curves which are in startlingly exact agreement. At room temperature on the other hand, λ 6402 and λ 5945 give curves of the same form though differing in magnitude, while the same is true for λ 6143 and λ 6334. The two pairs differ quite radically in the shape of the curve. λ 6163 and λ 6266 at room temperature differ somewhat but, as the error at these short half-life values is relatively large, it cannot be definitely said that they behave differently.

The error of the individual values of the points on the curves as stated in a previous paper is probably about 5%. This error is probably exceeded in the case of low values of the half-life, due, as stated above, to the occurrence of an error in the time settings which does not vary with their magnitude. Thus half-life values of less than 10×10^{-4} sec. are probably not accurate to closer than 10% of their value. It will be noted that these errors are far below the differences as shown between the various curves. Previous investigators (3) have suggested that a difference in half-width of the line might account for a difference in half-life as measured by different lines from the same state. Zemansky (4) has investigated this matter mathematically and has shown that a difference is to be expected, but that the effect will also show up as a departure of curves of $\log \frac{I}{I_0} = \frac{D_1 - D_2}{\gamma}$ plotted against time, from true exponentials. As shown by the plotted points, these measurements give exponential curves to the limits of their accuracy so that this explanation does not seem satisfactory. The alternative assumption that the "virtual oscillator" corresponding to each line has a real existence also seems rather radical, though less discordant with the more modern quantum theories than with the earlier ones.

Acknowledgment

The author wishes once again to express his thanks to the members of the technical staff of the laboratory, especially to Mr. Plaskett and Mr. Woodward whose assistance in the mechanical designing was invaluable. To Mr. Chappell also the author owes his thanks for much skilful work on the intricate glass flasks and discharge tubes.

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THE AIR AFTERGLOW AND ACTIVE NITROGEN¹

By J. K. ROBERTSON²

Abstract

In view of the lack of published photographs of the spectrum of the air afterglow, and of useful information which might be obtained relating to aurora discharges, the spectrum of this afterglow was photographed in the visible region. Excitation was accomplished by means of external electrodes used in connection with a Hartley oscillating circuit, a method which lends itself to the use of feeble excitations, and one, moreover, which does not seem to have been used hitherto. The spectrograms obtained show a continuous faintly banded region extending from below 4400 to 5000 or beyond, and a pure *continuum* in the yellow and red region with a distribution of intensity roughly corresponding to that shown by the yellow and red first positive nitrogen groups. Evidence is submitted that the part of the afterglow at the yellow-red end of the spectrum is due to nitrogen, not to oxygen, the source to which the continuous spectrum is generally ascribed. In an attempt to obtain further information regarding the nature of the entity emitting the air afterglow, by the addition of mercury vapor to the glowing gas, it was found that the glow was at once destroyed.

Introduction

Although afterglows in gases subjected to an exciting discharge had been observed even before (2, 3, 11 p. 249, 19) the work of Lewis (14) and Strutt (23), comparatively little has been done on the spectrum of the air afterglow. It is particularly noteworthy that there seem to be no published photographs of this spectrum. Most observers agree in describing the spectrum as continuous. Thus, Strutt (23) records a continuous spectrum extending from 4200 to 6700 and "doubtless into the infra-red." In later work by the same investigator (24) in connection with his study of active nitrogen, a mixture of oxygen and nitrogen in the same proportions as in air gave a greenish-yellow glow with a continuous spectrum extending to 4300 on the short wave-length side, with the other limit uncertain. A somewhat similar observation has been made by Constantinides (1) and by Kaplan (8). Working with ordinary air, Hagenbach and Frey (5) obtained the characteristic yellow nitrogen afterglow, whereas Herzberg (6) in a nitrogen-oxygen mixture, as well as in air, by varying the conditions, was able to obtain either the yellow nitrogen afterglow or a green afterglow with continuous spectrum, extending from red to blue, which he ascribed to oxygen. Stöck (22), who worked with air, confirmed Herzberg's work. Somewhat similar is the observation of Bernard Lewis (12, 13) who, working with a mixture of oxygen and nitrogen could, under certain conditions, obtain the α bands of nitrogen with a blue continuous spectrum as background.

Majewska (16), following up the work of Pienkowski (20), who obtained with air a "diffuse broad band", states that the air afterglow spectrum is continuous in the visible region but shows lines in the ultra-violet.

Kaplan (8, 9), working with air, was able to obtain either a blue afterglow showing an active nitrogen spectrum, or, with altered conditions, a greenish-yellow glow with continuous spectrum.

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Contribution from the Department of Physics, Queen's University, Kingston, Ontario.

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Although there is considerable evidence that the air afterglow showing a continuous spectrum is due to oxygen, this has not been conclusively proved nor has the exact nature and origin of the "active species" been explained. It seemed worth while, therefore, particularly in view of the lack of published photographs and of the possible information which might be obtained relating to aurora discharges, to photograph carefully the air afterglow with a mode of excitation not hitherto used. Previous observers have made use of ordinary induction-coil discharges, transformer discharges, as well as those obtained with damped high-frequency currents. In the work described in this paper, the writer used continuous high-frequency currents, obtained by means of a three-electrode transmitting tube, a method which lends itself particularly well to the use of feeble excitations.

It was considered also that it might be possible to pass glowing air into a vapor such as mercury and by so doing obtain valuable information by methods already used in the study of active nitrogen.

Experimental

The experimental arrangement which is shown in Fig. 1 needs little explanation. The upper part of the diagram shows the connections for the well-known Hartley oscillating circuit involving a S.W. 2-A Mullard transmitting valve fed by a 1500-volt generator. Loosely coupled to the coil L_1 is a second coil L_2 attached to external electrodes, either in positions AA' or BB' , between which the exciting discharge took place in air entering the apparatus through the control valve. No precautions were taken to dry or to purify the air in any way. To produce intermittent discharges a relay R was used which made and broke the grid-leak circuit by means of a commutator on the shaft of a motor rotating the sectored disk S . The discharge was flashed on and off about $3\frac{1}{3}$ times per second, the "on" period being about $\frac{1}{3}$ of the total time. For most of the work the wave-length of the high-frequency current was about 36 m.

The afterglow was photographed through the quartz window W with a

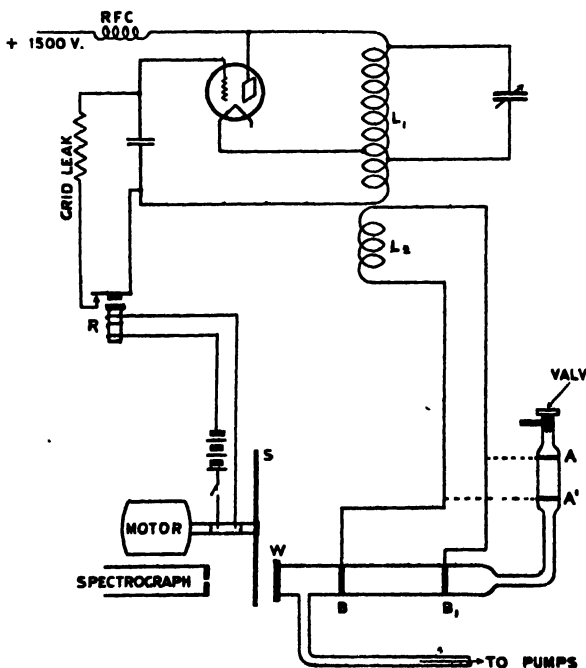


FIG. 1. Diagram of circuit used to excite discharge in air and to photograph afterglow.

double-prism Ladd spectrograph which had previously been found extremely useful for sources of feeble intensity. Ilford special rapid panchromatic plates were used.

Some preliminary work was done to determine the most favorable conditions for obtaining a good afterglow. As the walls of the containing vessel undoubtedly have a marked influence on the afterglow (12), observations were made with three tubes of different sizes. The first was cylindrical and about 4 cm. in diameter; the second cylindrical, about 10 cm. in diameter; the third spherical, the radius being 13 or 14 cm. It was found that, although glows lasted somewhat longer in the largest vessel than in the smallest, they were of less intensity. Moreover, with both the largest vessel and that of intermediate size it was not so easy to obtain satisfactory glows. Most of the work, therefore, was carried out with the aid of cylindrical tubes 3 or 4 cm. in diameter.

To produce satisfactory afterglows, it was found that the air should not be stagnant. Indeed, during the period of several days which was necessary for a photograph of the spectrum, not only was air continually pumped through the apparatus (during actual exposure) but, in addition, each night, after shutting off the pumps, air was left in the system at atmospheric pressure. Somewhat similar is the observation of Majewska and Bernhardt (17), who found it necessary, in order to obtain a good afterglow, to put their tube in a "vorbereitet" condition by pumping air through or by washing out with air.

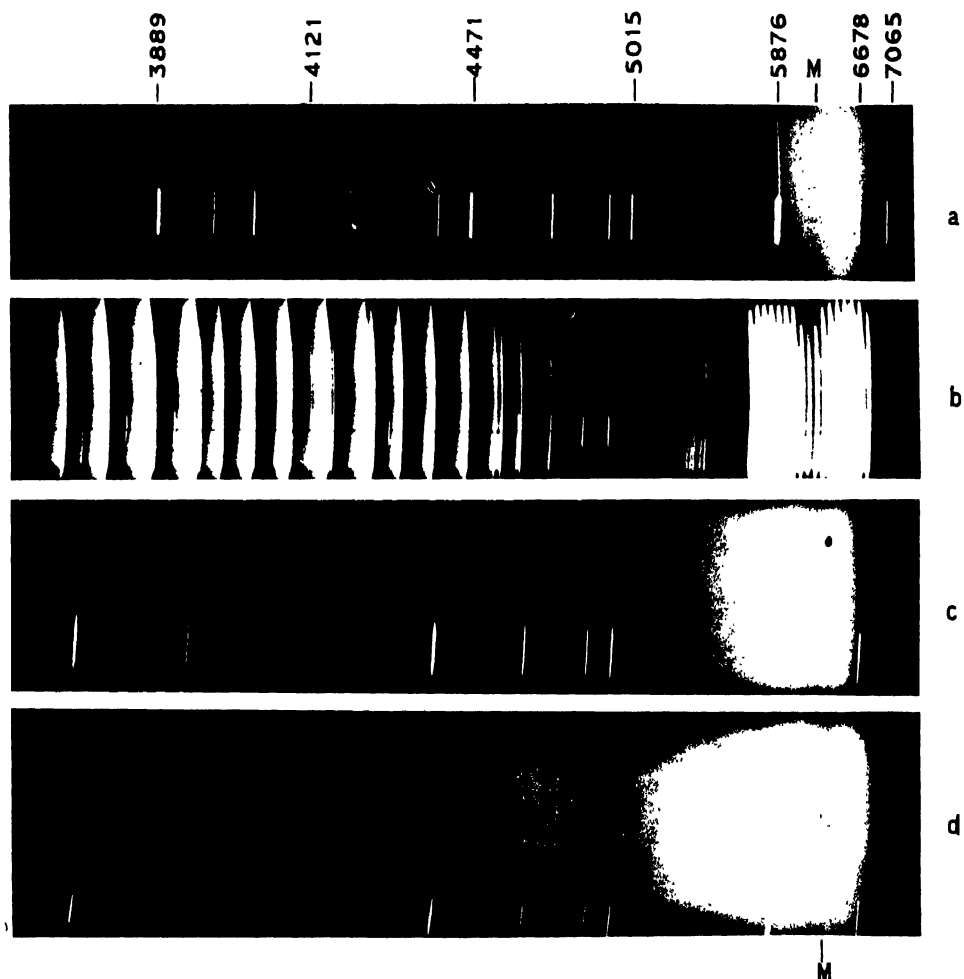
The intensity of the afterglow seemed, if anything, to improve with the length of time the discharge had been taking place, another indication, no doubt, of the sensitiveness of the afterglow to the conditions of the walls. Just what change is brought about in the surface by the electrical action, it is difficult to say, but that it is important is further emphasized by the statement of Kaplan (10) that active nitrogen was obtained by a new method which involved a two-weeks preliminary running of an uncondensed discharge in nitrogen.

Afterglows could be obtained at pressures ranging from about 1.25 mm. to as low as 0.03 mm. At the lower pressures, although the afterglow was sometimes of longer duration, it was less intense, and the predominantly greenish color changed to a somewhat whitish shade, *e.g.*, at pressures such as 0.12 mm. At the higher pressures, *e.g.*, 0.8 mm., the color was for the most part green, though sometimes greenish-yellow. However, one observation at 0.61 mm. showed the tendency to white which normally appeared at lower pressures. When the spectrograms *c* and *d* reproduced in Plate I were photographed the color was green and the pressure about 0.7 mm.

The direct discharge which gave rise to a good afterglow had a characteristic appearance. Between the electrodes it was pink, but on either side, a greenish glow of about the same shade as the subsequent afterglow extended for some distance.

For some reason, the direct discharge could be obtained more readily in the tube with electrodes *BB'* (tube of diameter about 2.4 cm. and length 42 cm.) than in the one with electrodes *AA'* (tube of diameter 4 cm. and length 15 cm.), although, with careful manipulation, discharges and satisfactory afterglows

PLATE I



Spectrum a: active nitrogen + calcium vapor; exposure, 3 hr.

Spectrum b: direct discharge in air; exposure 8 min.

Spectrum c: air afterglow; exposure 29 hr.

Spectrum d: air afterglow; exposure 29 hr.

could be obtained in the latter. Indeed, under favorable conditions with this tube, the green extension of the direct discharge extended so that it filled the tube with the quartz window, where, of course, it persisted on the removal of the exciting potential. As the afterglow was of short duration, never lasting for longer than seven seconds, and as such satisfactory glows were obtained when electrodes *AA'* were used, the spectrograms were taken with the direct discharge in the tube to which they were attached.

Results

In the accompanying plate, Spectrum *b* is that of the direct discharge. The marked intensity of the first and second positive bands of nitrogen will be noted. Both *c* and *d* are spectra of the afterglow, the exposure for each being 29 hr. It may not be amiss to state that between the exposures the apparatus was partly dismantled and a new tube installed in place of the old one in which the discharge had been taking place. It will be noted that both spectra show a continuous faintly banded region extending from below 4400 to 5000 or beyond, as well as a *continuum* beginning in the region corresponding to the green group of the first positive band system and extending to the long wavelength side of the red group. If the detail of individual bands be neglected, the intensity distribution in this truly continuous region is closely parallel to that shown by the green, yellow and red first positive groups in the direct discharge. Although the reproduction does not show it well, in both *c* and *d* there is a pronounced minimum corresponding in position to the region of minimum intensity between the yellow and red groups in the direct discharge.

(At the lower edge of Spectrum *d*, the presence of second positive bands as well as the typical structure of the red first positive group will be noticed. This is undoubtedly due to a faulty adjustment of the rotating sector which allowed a trace of light from the direct discharge to enter one end of the slit of the spectrograph. No sign of either second positive bands or of structure in the red end of the afterglow appears in the upper half of this spectrum, nor is there any sign of either in Spectrum *c*.)

Before discussing the above spectra, it is desirable to refer briefly to the results obtained when mercury vapor was added to the gas in the tubes. For this purpose a small amount of mercury was inserted in a side tube attached to the long tube with the quartz window. Before warming the mercury, the direct discharge showed the characteristic pink color with the diffuse green extension. On heating the mercury the green glow disappeared entirely and suddenly. Difficulty was subsequently experienced in obtaining a good afterglow even in the presence of mercury at room temperature. By cooling the side arm tube with solid carbon dioxide most of the mercury could be collected in that place, but even under those conditions, after the discharge had been running for an hour, the afterglow was of less intensity than at the outset. It may be concluded, therefore, that the presence of mercury vapor in appreciable amounts inhibits or destroys this type of afterglow.

Discussion

Although Strutt in his early work on the air afterglow ascribed the origin to the combination of ozone with nitric oxide, and although recent observers have stated that the green afterglow with continuous spectrum is due to oxygen, it seems to the writer that the origin of that part of the afterglow giving rise to the yellow and red end of the spectrum must be related to nitrogen. The evidence for this is found in the fact that the spectrum of the exciting discharge is that of nitrogen, as shown in Spectrum *b*, but most of all, in the information yielded by an examination of Spectrum *a*. This is a reproduction of one of several plates taken in this laboratory some four years ago by J. H. Findlay (4) in connection with his investigation of certain metallic spectra excited by active nitrogen. It will be noted that, allowing for differences in exposure, there is almost an exact agreement between Spectrum *a* and Spectra *c* and *d* in so far as the yellow-red end is concerned. Note, for example, the position of the region of minimum intensity (marked *M*) in the spectra. In Spectra *c* and *d* the yellow and red bands seem to be truly continuous but a close examination of the red band in Spectrum *a* reveals the presence of a structure which seems to be identical with that of the red group of the first positive bands of nitrogen.

Now, in Findlay's work, streaming active nitrogen, generated by a strong electrodeless discharge in nitrogen (not air) was passed into a tube in which, for Spectrum *a*, metallic calcium was strongly heated. The resulting spectrum, reproduced in *a*, showed sodium (a calcium impurity or perhaps from the heated Pyrex) lines and the yellow and red apparently continuous region to which attention has been called. This same bit of apparently continuous spectrum was observed by Findlay on another calcium plate as well as on one obtained when mercury vapor was added to active nitrogen. A six-hour exposure on the active nitrogen itself, however, showed only the α collection of first positive nitrogen bands. There seems no doubt, therefore, that that part of the air afterglow giving rise to the yellow and red end of Spectra *c* and *d* is due to nitrogen, not to oxygen. There is, of course, the possibility that oxygen might have been given off by the heated calcium and mercury and excited by active nitrogen but, in an exposure lasting several hours, one would think that after the first heating, most of the occluded oxygen would have been removed. Oxygen bands may be obtained in the red-yellow region but, according to the work of Lockrow (15), the potential necessary to excite these is over 20 volts. As active nitrogen has given rise to no lines requiring an excitation potential greater than 9.5 volts the yellow-red described in this paper could have no connection with these oxygen bands.

That active nitrogen can be obtained by discharges in air has been established by the work of Herzberg (6), Kaplan (8), and Mukherji (18), but the glow observed does not seem to be of the same type as that described in this paper. Certainly that obtained by Kaplan is different, for he states that his afterglow showed typical α bands of nitrogen as well as the β and α bands of nitric oxide.

It seems to the writer that there is evidence in favor of the view that this yellow-red afterglow results from a modified, perturbed emission of nitrogen

bands brought about by the action of some agency on excited nitrogen molecules. In Findlay's plate the perturbation is not enough to completely remove the band structure; in the air afterglow, it is more pronounced and the structure disappears. The phenomenon is probably not unrelated to the modification in the distribution of intensity in the α group of active nitrogen, observed by Rayleigh (21) and by Herzberg (7). Rayleigh obtained a marked shifting of intensity to the red group by the addition of helium, whereas Herzberg, by cooling with liquid air, observed a shift in the opposite direction.

In Findlay's work it will be recalled, the continuous band was obtained only when active nitrogen encountered a foreign vapor. Just what the process is that perturbs the vibrational levels is difficult to see, but the evidence seems to favor strongly some such process rather than the ascribing of the appearance to a reaction between ozone and nitric oxide, or to a recombination spectrum of oxygen.

It is probable that the remainder of the afterglow spectrum shown in *c* and *d* *i.e.*, the blue-green portion with faint structure, is due to oxygen, as there is no trace of this part in Spectrum *a*. The writer had hoped to photograph the afterglow from pure oxygen but circumstances prevented him doing so, and other work will not permit this being done immediately.

Acknowledgment

Almost all the experimental work described in this paper was done by Mr. Keith MacKinnon, M.Sc., whose knowledge of high-frequency circuits was of great value. The writer's thanks are also due Mr. Stewart Marshall, B.A., and Mr. W. H. Henderson, M.A. who made some preliminary observations on the afterglow, and to Mr. C. W. Clapp, B.Sc., who did the photographic enlarging. The 1500-volt motor-generator set used in connection with the oscillating circuit is the property of the National Research Council of Canada. For permission to continue using this piece of apparatus for research purposes, the writer can only express his sincere appreciation and grateful thanks.

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A PRELIMINARY INVESTIGATION OF THE MANNER OF REMOVAL OF LIGNIN FROM SPRUCE IN CONCENTRATED SODIUM HYDROXIDE SOLUTIONS¹

By L. S. MACKLIN² AND O. MAASS³

Abstract

An apparatus and method have been devised for studying the cooking of wood with sodium hydroxide solutions. The apparatus was so designed that the amount of wood was smaller, and temperature, pressure and concentration of cooking liquor could be controlled more accurately, than heretofore. Wood samples of known bone-dry weight were then treated under fixed and reproducible conditions. The cooking of small spruce chips at 160° C. has been investigated for time periods up to three hours and using 20, 25, 29, 32 and 40% sodium hydroxide solutions, a concentration region not previously investigated. The pulp produced contained a certain amount of lignin changed so as to be readily soluble in dilute alkali. This together with lignin contained in the final cooking liquor was estimated. The rate of solution of lignin in the liquor was found to vary as the inverse cube of the sodium hydroxide concentration in the range examined. A tentative hypothesis based on lignin being a lyophilic colloid has been put forward to account for the following generalizations which could be made with regard to the data obtained. (a) Lignin cannot be dissolved until it is changed into a "dilute alkali-soluble form". (b) Increase in concentration of sodium hydroxide acts in two ways; first, in increasing the amount of this form and, second, in preventing it from leaving the pulp proper.

Introduction

In the commercial soda process for the manufacture of wood pulp, cooking liquor solutions containing 5 to 10% of sodium hydroxide by weight are employed. Investigations, such as those of Sutermeister (6), into the influence of variable factors showed that increase in the alkali concentration of the liquor is accompanied by decrease in the yield of pulp. However, in these studies concentrations much above 15% of sodium hydroxide were not tried, since even in this region the yield of pulp was too low to be profitable.

In 1930 Ross (3) showed that wood could be pulped satisfactorily with cooking liquor containing 40% of sodium hydroxide by weight. The use of alkali of this concentration was investigated further by Ross and Adlington

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(1, 4). The pulp yields were quite good, showing that the influence of concentration upon yield must suffer a change of sign at some value of the concentration intermediate between the lowest and the highest. Ross (3) found however that cooking with 40% alkali gave results quite different from those obtained with solutions of the usual strength. Apart from the differences in character of the pulps produced, there was a marked difference in the manner in which the lignin was removed from the wood.

Thus in the ordinary process, with dilute alkali, the lignin is removed continuously during the cooking. If the wood residue is taken from the liquor and thoroughly washed to remove sodium hydroxide, it may be dried and analyzed by one of the empirical methods for estimating lignin. Provided the cooking treatment has been sufficiently severe, say six hours at 170° C., the pulp is found to contain very little lignin. If the cooking liquor, a dark colored solution, be acidified, a heavy brown precipitate appears and may be filtered off, washed and dried. This amorphous substance, although undoubtedly different from lignin as present in the original wood, corresponds in amount, within a few per cent, to the loss of lignin from the wood. It is called alkali-lignin, and has been the subject of much research.

With 40% sodium hydroxide, Ross (3) obtained the following markedly different effects. In the first place the cooking liquor was pale yellow in color instead of red-brown. Deep coloring, however, was found in the water with which the pulp was washed. The pulp contained very little lignin, as did also the cooking liquor. On acidification of the liquor no precipitate was thrown down. The missing lignin was found in the alkaline wash water. Ross remarked, "It was found that the ligneous incrustations were insoluble in the cooking liquor, though changed to the extent that they were readily dissolved by liquor below 20 percent NaOH by weight at any temperature."

A search of the literature failed to reveal any reference to the use of sodium hydroxide solutions of concentrations intermediate between 20 and 40%. Ross considered that at some concentration between that used in the commercial process and 40%, a sharp transition from the usual behavior to that typical of 40% solutions would be found. The present investigation was undertaken with the purpose of obtaining more complete data on the influence of concentration upon the manner of removal of the lignin.

In an investigation of the soda cooking reaction the chief variable factors which may be considered are temperature, concentration of sodium hydroxide, and time of cooking. In the present work it was found possible to study only one temperature, 160° C., throughout the concentration range from 20 to 40% sodium hydroxide, for various time periods up to three hours.

Apparatus

The charges of wood were cooked in a small digester of special design. It was desirable to use a small digester in this work so that at the start of an experiment the required cooking temperature could be attained very rapidly. The long "heating-up" period of commercial cooks was thus eliminated. At the same time it was necessary to maintain the alkalinity of the cooking

liquor as nearly constant as possible during an experiment. To this end, fresh liquor was introduced into the digester at frequent intervals, while the spent liquor was withdrawn at a corresponding rate.

The details of the apparatus may be understood more clearly by referring to the diagrams. The cell or digester (Fig. 1) in which the wood was cooked was formed from an Allegheny steel tube (marked 1 in the diagram): internal diameter, 2 in.; external diameter, $2\frac{1}{2}$ in.; height, $1\frac{7}{16}$ in. This tube was clamped between two $\frac{1}{4}$ -in. Allegheny steel plates (2, 2). Each of these plates was drilled and tapped to take a $\frac{1}{8}$ -in. short iron nipple (3, 3). By means of $\frac{1}{8}$ -in. iron tees (4, 4) and steel compression fittings (5, 5), two copper tubes (6, 6) were made to connect with the opening through each plate. The couplings of the compression fittings were turned from $\frac{1}{2}$ -in. mild steel rod, bored to take $\frac{1}{8}$ -in. copper tubing and threaded for $\frac{1}{8}$ -in. I.P.S. caps. The caps were also bored to take $\frac{1}{8}$ -in. copper tubing. Instead of cones, ordinary graphite packing was wrapped around the tubes.

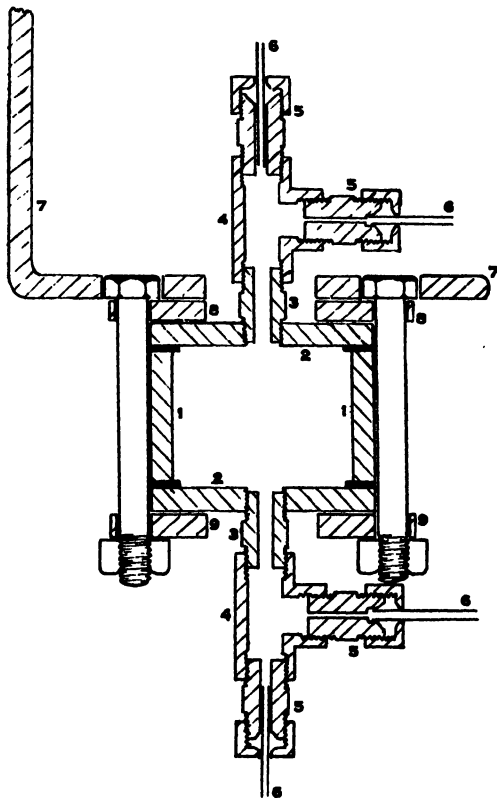


FIG. 1. Cross-section diagram of the digester.

The cell was formed from these parts by clamping the two plates over the ends of the tube, as shown in the diagram. Lead gaskets were inserted between the steel surfaces, so that the cell could be made tight. The cell was clamped together and supported in the following way. A heavy angle bracket (7) made from $\frac{1}{4}$ -in. iron plate, $3\frac{1}{2}$ in. wide, was bolted firmly to the wooden frame of the apparatus. The horizontal part of this bracket was 5 in. long, and made therefore an immovable flat plate, 5 in. long by $3\frac{1}{2}$ in. wide by $\frac{1}{4}$ in. thick. To the under side of this plate a steel disk (8), $3\frac{1}{4}$ in. in diameter by $\frac{1}{4}$ in. thick, was fastened by four $\frac{3}{16}$ -in. bolts. These bolts are not shown in the diagram. The plate and disk were then bored to take four $\frac{3}{8}$ -in. bolts $3\frac{1}{2}$ in. long, the hexagonal heads being countersunk through the upper plate. These holes were placed symmetrically on a circle 3 in. in diameter. A large hole, $1\frac{1}{4}$ in. in diameter, was also bored through the plate and disk, in a position central with regard to the four smaller holes. A second $3\frac{1}{4}$ -in. disk was made (9), and bored with a $1\frac{1}{4}$ -in. hole in the centre and four $\frac{3}{8}$ -in. holes at the edges. This disk was then fitted on to the ends of the four heavy bolts. Then the upper

cell cover was fitted under the plate and disk, the tee and compression fittings protruding upwards through the large hole. The lower cell cover was placed similarly with regard to the lower disk, and the cell tube then fitted between the two cell covers. Hexagonal nuts were screwed onto the bolts, and the three parts of the cell were drawn tightly together. Once assembled, the dismantling and reassembling of the cell were very simple procedures, since they required only the removal and replacement of the cell tube.

To facilitate the charging of the cell with the small chips of wood used in this work, a small copper gauze cage was made and fitted permanently into the cell tube. The cage was cylindrical in shape, the sides being made of finely perforated copper sheet, and the bottom being a coarse copper wire netting. The chips of wood were packed into this cage and covered with a circular piece of metal gauze.

A diagram of the various connections leading to and from the cell is given in

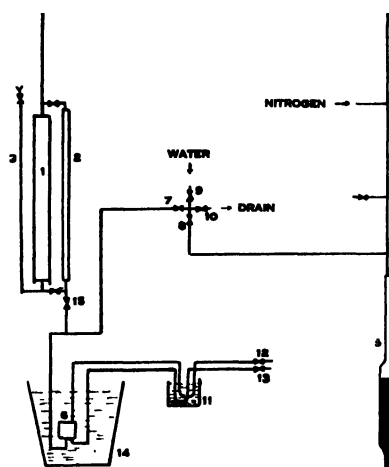


FIG. 2. *Diagram of pressure connections.*

Fig. 2. A reservoir (1) capable of holding sufficient cooking liquor for the performance of one experiment was made from a piece of 2-in. black iron pipe, 23 in. long. This was fitted with a gauge glass (2) and a side tube (3) to facilitate filling. Since it was necessary to introduce the cooking liquor into the cell under pressure, the top of the reservoir was connected by a pipe line (4) to a cylinder of compressed nitrogen. The pressure was regulated by a reducing valve on the cylinder, and was measured by a simple compression manometer (5). The bottom of the reservoir was connected to one of the copper tubes from the lower side of the cell (6). This inlet was connected also to the water-main, to the nitrogen line, and to the drain (valves 7, 8, 9, 10). The other copper tube from the lower side of the cell, and one from the upper side, were led

through a cold water bath (11) and served as outlets for the cooking liquor (valves 12, 13).

The temperature of the cell was controlled by having it immersed in a thermostatically controlled oil bath (14). The heating, stirring, and regulating were done electrically.

A galvanized pail formed the container of the bath, and this was suspended by ropes which passed over pulleys and carried a counterpoise. The bath was thus easily raised and lowered.

Experimental

The wood was used in the form of small flat chips, about 2 cm. long in the fibre direction, 1 cm. wide and 2 mm. thick. This form was found to be more

satisfactory than coarse shavings, since about ten grams of chips could be packed into the cell, as compared with only seven grams of shavings. Thin cross sections, say 2 mm. in fibre length, were also tried, but were found to be unsatisfactory on account of the difficulty of straining the short fibred pulps produced by them. A considerable quantity (a few pounds) of these chips was made up at one time from a piece of black spruce. The moisture content of these chips was determined by drying a weighed sample at 105° C. for several days, and re-weighing. The supply of chips was kept in a stoppered bottle, and the moisture content of the wood was found to change very little: in seven weeks it decreased from 6.42 to 6.10%. The sample of wood to be used in an experiment was weighed out in the ordinary condition, without drying, and the actual bone-dry weight calculated from the known moisture content.

The caustic solutions were made up from ordinary c.p. sodium hydroxide sticks, the concentration being determined in each case by titrating a weighed sample with normal hydrochloric acid.

In making an experimental "cook", the following procedure was used.

The weighed sample of wood (about ten grams) having been introduced into the cell and the clamping bolts made tight, valves 12, 13 and 15 were closed and a high nitrogen pressure, say 120 pounds, applied through valves 7 and 8 (Fig. 2). Any rapid leak in the cell or cell connections was then readily found, and could be repaired. The nitrogen pressure was released and the reservoir filled with sufficient soda liquor for the performance of the experiment in hand.

The oil bath was heated up to the required temperature before the cell was immersed in it. This was done by raising the pail a few inches from the floor and heating it with Meker burners. It was found necessary to have the bath about 5° C. hotter than the proposed temperature of the experiment, to compensate for the cooling effect of plunging the cold cell into the oil. Generally half an hour was required to raise the temperature to the desired point.

Five or ten minutes before the experiment was to start, valves 7, 12 and 15 were closed, and the cell was evacuated by connecting outlet 13 to the water pump. This evacuation was performed merely to prevent the occurrence of air bubbles in the spaces between the chips when the liquor was forced into the cell.

The bath was then run up and the cell immersed in the oil; the heater and stirrer were started and the burners extinguished. After a lapse of one minute, in which time the cell was assumed to have acquired the temperature of the bath, valve 13 was shut and liquor introduced through valve 15 under a nitrogen pressure sufficient to prevent boiling.

From this point, quantities of the liquor (25 to 50 cc.) were withdrawn at five-minute intervals, so as to keep the alkalinity of the cooking liquor as close as possible to that of the original solution. The alkalinity of the effluent liquor was determined approximately from time to time by titrating a 1-cc. sample with normal hydrochloric acid. If the titre fell markedly below that of the original solution a larger quantity of liquor was withdrawn from the cell, and so on. The effluent liquor was collected in a covered flask.

At the end of the experiment valve 15 was closed, the heaters and stirrer turned off, and the oil bath lowered away from the cell. Then, as rapidly as possible, the pail of oil was replaced by one of cold water, and the latter run up until the cell was completely immersed. In a few minutes the cell was quite cold, and the pail of water was removed. The liquor in the cell was then blown out as follows. On opening valves 12 and 13 a little liquid flowed out of its own accord. These valves were then shut and nitrogen introduced into the cell under pressure. On shutting valve 7 and opening 13 the gas in the cell was able to expand and force the liquid through the outlet.

The cell having been emptied of liquor so far as possible, it was dismantled and the pulp removed to a large beaker. The experiment proper was now complete. Investigation of the pulp and liquor remained to be carried out, and this was done as follows.

The pulp, being still associated with an excess of cooking liquor, had to be treated, before drying, by some method which would remove the alkali and at the same time have as little effect as possible on the lignin content of the pulp. Now since, as Ross (3) showed, the treatment of wood with strong alkali solutions leaves a considerable proportion of the lignin in a changed condition, so that it is soluble in cold dilute alkali, it was necessary to avoid if possible the dilution of the alkali in contact with the pulp. The only way to do this, apparently, was to transform the sodium hydroxide into a sodium salt which could then be washed out with water. The pulp in the large beaker was therefore stirred up with a mixture of 30 cc. of concentrated hydrochloric acid and 700 cc. of water. The stirring was accelerated by the use of a small "Dumore" disintegrator (Type 6). This procedure, however, gave rise to a complicating factor, in that the neutralization was accompanied by the appearance of a white gelatinous precipitate. This precipitate was readily separated from the pulp by straining through a Büchner funnel, which retained the pulp, but allowed the precipitate to pass through with the liquid. The pulp was removed from the funnel and stirred up with about 700 cc. of hot water. It was then collected on the Büchner funnel and washed repeatedly with hot water. This washing generally resulted in further separation of precipitate from the pulp. The acid filtrate and washings were combined, giving 2 to 3 litres of liquid, and set aside for investigation of the precipitate. The pulp was placed in a weighed aluminium container and dried at 105° C. for several hours.

The precipitate in the acid wash, although very fluffy in appearance, settled rapidly to the lower part of the container. In one of the first experiments a small quantity of this precipitate was transferred by means of a tube to a glass slide, and examined under the microscope. Apart from a little cellular debris and a few stray fibres the mass had no structure discernible even with the oil immersion lens. Hertzberg's solution stained the cellular matter blue, the amorphous matter yellow, indicating that the former was cellulose, the latter lignin. The precipitate was not stained red by phloroglucinol, however, and was therefore not "unchanged lignin". Taking into account the manner of formation and the appearance of the precipitate it seemed reasonable to suppose that it was simply alkali-lignin which had been clinging loosely to the fibres

and which was separated from them when the fibres were torn apart in the disintegration of the pulp. In any case, whatever was the source of this substance, it was necessary to determine the amount of ligneous material in it, and this was done in the following way.

The acid washings and precipitate were heated on the steam bath until the volume had been reduced to about 500 cc. This generally took about 24 hr. and rendered the precipitate darker and somewhat granular. The hot liquid was then poured through a fluted filter paper (11 cm.) in an ordinary funnel, and the precipitate washed with a little water. After draining, the paper and precipitate were transferred to a small beaker, and the lignin content estimated by the method of Ross and Potter (5).

The pulp, after thorough drying, was weighed, and from the calculated bone-dry weight of the original wood the percentage yield obtained. Samples of the pulp were then analyzed for lignin, and for solubility in 1% sodium hydroxide. The extraction with a 1% alkali dissolves out any "changed lignin", *i.e.*, lignin which, although not dissolved by the cooking, is nevertheless altered in such a way that it may be dissolved by dilute alkali, even in the cold. The extraction was carried out in the standard way, as described in text books on wood. To determine how much "changed lignin" was contained in the pulp, a sample of the extracted pulp was then analyzed for lignin in the usual way. The difference between the lignin content of the original pulp and that of the extracted pulp gave, therefore, the quantity of "changed lignin" in the pulp.

There now remained for consideration only the cooking liquor. This was always of a yellow color, the shade being deeper the lower the concentration of alkali. It always contained, however, a considerable amount of thick dark green precipitate, which appeared only after standing, and which collected at the top of the liquid. The precipitate dissolved in acids, and was consequently assumed to be non-ligneous in nature. As the scope of this work required only a knowledge of the quantity of alkali-lignin in the liquor, no further attempt was made to determine the character of this precipitate.

The liquor was thoroughly shaken and poured into a measuring cylinder. The total volume was recorded, and an aliquot portion, 100 cc., pipetted into a beaker and diluted with an equal volume of water. Concentrated hydrochloric acid was then added until the mixture was strongly acid. At the neutral point the solution became quite clear and nearly colorless. The absence of precipitate at this stage, however, did not indicate that there was no alkali-lignin present, for when the solution was heated for a few hours in a covered beaker on the steam bath, the liquid became cloudy and finally a gelatinous light brown precipitate was formed. This was filtered off, washed, and analyzed for lignin in the same way as the precipitate from the acid wash. From the result, the quantity of lignin which could have been obtained from the whole cooking liquor by this method was calculated, and this amount taken as representing the lignin actually dissolved in the cooking.

In brief, the performance of such an experimental cook and its attendant analyses provide the following data:

A sample of wood, the bone-dry weight of which is known, has been treated under definite temperature and pressure conditions for a definite length of time with liquor of approximately known strength. As a result of this treatment a quantity of pulp has been produced containing a definite amount of lignin. Of this lignin a certain quantity has been so changed that it is readily dissolved by dilute alkali: this fraction is known. At the same time a certain quantity of lignin has been dissolved from the wood by the cooking liquor. Finally, an amount of lignin has been split off from the pulp in the washing process; this is changed lignin, and should be considered, presumably, along with the changed lignin in the pulp.

Such a set of data was obtained for each of 20 different experimental cooks. In this series, at the one temperature, 160°C. , a range of four different time periods and five different concentrations of sodium hydroxide was covered. Not every experiment was repeated, but a number of repetitions were made, and the results of these generally agreed fairly well with those of the original experiments. The best agreement was obtained with the data for the lignin content of the extracted pulp and the amount of lignin dissolved in the liquor. As will be seen shortly, it was these two quantities which were found to display definite relations with concentration and time of cooking.

Results

The analytical results are given in Table I. All values are expressed in relation to the bone-dry weight of the original wood taken as 100%. Included with them are certain combinations of the original data:

The "total cooking" or "total delignification", D , represents the difference between the lignin content of the original wood and that of the pulp after extraction with dilute alkali.

The ratio of the amount of the lignin in the liquor, L , to the total delignification, D , is interesting. It represents the fraction of the lignin, available for solution, which has actually been dissolved.

The ratio $D/(D-L)$ was found to display interesting variations with variation in the concentration of sodium hydroxide.

Discussion of Results

If now the values of the lignin in the liquor, L , are plotted against the corresponding cooking times, the graph shown in Fig. 3 is obtained. Within the limits of experimental error, the points lie on a straight line

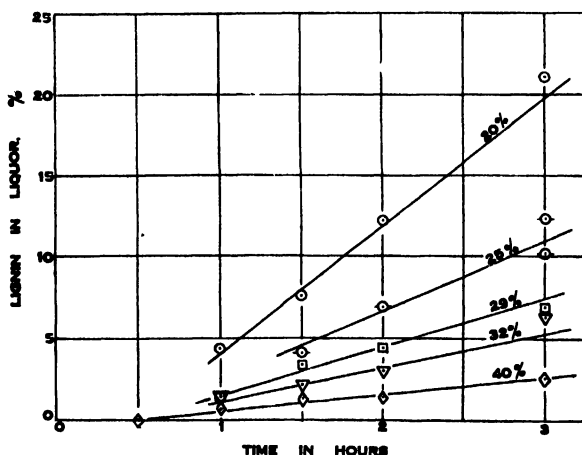


FIG. 3. Graph showing the relation between the quantity L and the cooking time for different concentrations of sodium hydroxide.

TABLE I
EXPERIMENTAL RESULTS

Time of cooking, hr.	Concentration of NaOH in cooking liquor, %				
	20	25	29	32	40
Lignin in original pulp, %					
1	20.8	17.1	15.4 17.1	15.4 17.2	13.9
1.5	17.1 10.4	11.7	14.9	18.0	9.1
2	10.5	12.3	8.5	11.3	10.4
3	6.7	6.3 7.4	11.0	12.1	9.6
Lignin in acid wash, %					
1	1.2	5.4	11.6 6.0	5.4 6.3	9.2
1.5	3.3 5.4	6.2	5.6	3.6	12.7
2	0.8	3.1	10.3	7.9	8.4
3	2.1	9.9 7.8	5.2	6.1	7.7
Lignin in liquor, % (L)					
1	4.3		1.4 1.4	1.7 1.4	0.7
1.5	7.6 7.7	4.0	3.3	2.1	1.3
2	12.2	6.9	4.3	2.9	1.4
3	21.0	10.9 10.2	6.9	6.3	2.5
Lignin in extracted pulp, % (e)					
1	13.0	10.5	8.6 9.4	4.9 5.9	1.9
1.5	6.3 5.5	6.1	4.4	3.6	1.8
2	4.0	4.0	3.5	1.9	1.0
3	1.7	2.3 2.7	0.9	1.2	0.5
Total cooking, % (D), (29-e)					
1	16.0	18.5	20.4 19.6	24.1 23.1	27.1
1.5	22.7 23.5	22.9	24.6	25.1	27.2
2	25.0	25.0	25.5	27.1	28.0
3	27.3	26.7 26.3	28.1	27.8	28.5
Ratio L/D					
1	0.268		0.070	0.066	0.026
1.5	0.333	0.175	0.134	0.084	0.048
2	0.488	0.276	0.169	0.107	0.050
3	0.770	0.396	0.246	0.228	0.088
Ratio D/(D-L)					
1	1.37		1.07	1.07	1.03
1.5	1.50	1.21	1.15	1.09	1.05
2	1.95	1.38	1.20	1.12	1.05
3	4.35	1.66	1.32	1.29	1.12

for each of the different concentrations. Therefore the rate of solution of lignin in the liquor is a constant for any given concentration of sodium hydroxide.

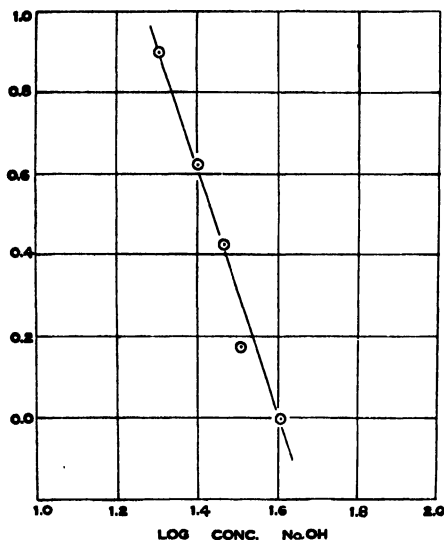


FIG. 4. Graph showing the logarithmic relation between the rate of solution of lignin and the concentration of sodium hydroxide.

be a conversion of the original lignin into a form which is completely soluble in dilute alkali. This changed lignin is then gradually acted upon by the liquor and goes into solution or fine suspension. The rate at which the first reaction proceeds is much higher at higher concentrations of sodium hydroxide. This can readily be seen by plotting total delignification against time of cooking, as shown in Fig. 5. On the other hand, the rate at which the second reaction proceeds, *i.e.*, the rate at which the changed lignin is dispersed in the liquor, must decrease very rapidly with increase in concentration of sodium hydroxide.

If the solution of lignin does take place in two stages, then the amount of lignin finally obtained in solution should be considered as a fraction of the amount changed. If this

In fact, if the logarithm of the rate (the logarithm of the slope of the line) is plotted against the logarithm of the concentration, a straight line is obtained, as shown in Fig. 4. This indicates that the rate of solution of lignin in the liquor, dL/dt , is related to the concentration of sodium hydroxide, c , by the equation

$$dL/dt = K_1 c^n$$

The value of n is found to be very nearly -3 (-3.05 as calculated).

The fact that the amount of lignin actually dissolved in the liquor may be only a small fraction of the total delignification, and the fact that the amount of lignin going into solution increases with time, even when the total delignification is complete, suggests that the solution of the lignin in the liquor takes place in two stages. Thus the first reaction may

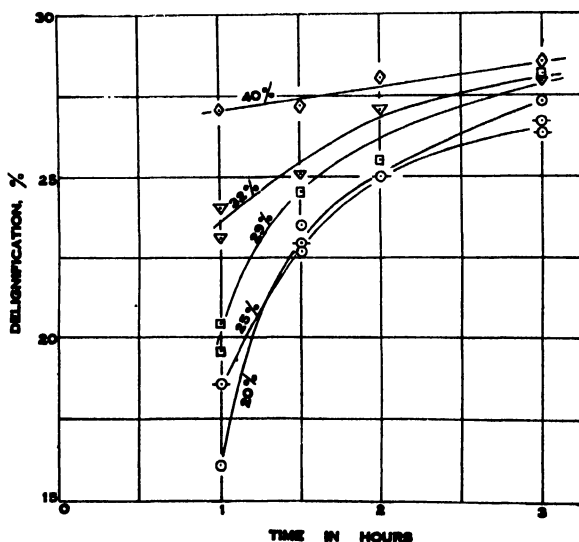


FIG. 5. Graph showing the relation between the quantity D and the cooking time for various concentrations of sodium hydroxide.

fraction, the amount of lignin in the liquor divided by the total delignification, be plotted against cooking time, the graph shown in Fig. 6 is obtained. Again, within the limits of experimental error, straight lines are obtained for the different concentrations of sodium hydroxide. Now in the case of the values of L considered apart from those of D , it may be seen in Fig. 3 that the straight lines appear to converge to a point on the time axis at or near the 0.5-hour period. This suggests that no lignin goes into solution in the liquor during the first half hour of cooking, regardless of the concentration of sodium

hydroxide. Such a time lag might be explained as representing the time required for complete permeation of the wood with liquor and opening up of the

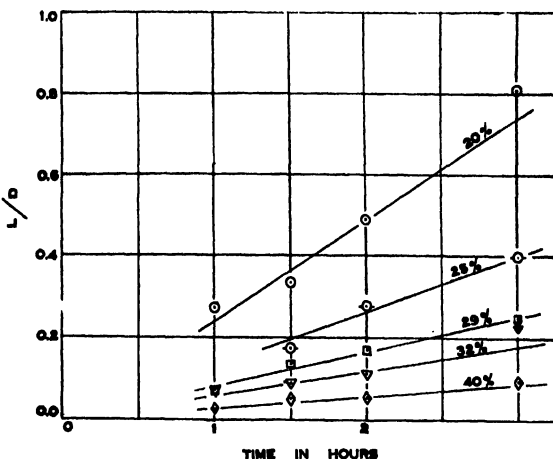


FIG. 6. Graph showing the relation between the ratio L/D and the time of cooking, for different concentrations of sodium hydroxide.

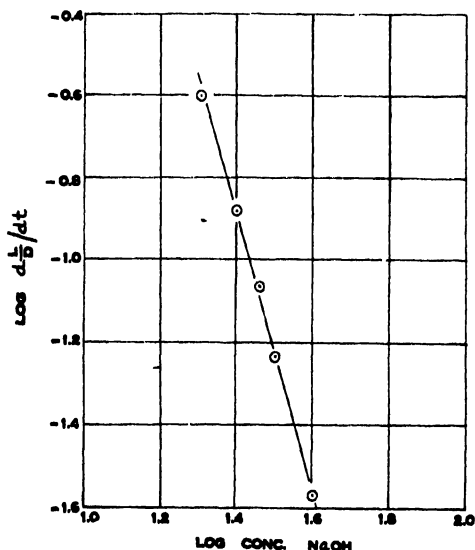


FIG. 7. Graph showing the logarithmic relation between $\frac{dL}{D}/dt$ and the concentration of sodium hydroxide.

concentration suggests that the second reaction is a fine colloidal dispersion rather than a true solution of products formed by chemical decomposition of the lignin.

wood structure. On the other hand the straight lines obtained by plotting L/D against t (Fig. 6) appear to converge toward the origin. This confirms the idea that lignin cannot be dissolved in the liquor until it is changed into the "dilute alkali-soluble" form. Now if the slopes of these lines be calculated, and the logarithms of the slopes plotted against the logarithms of the sodium hydroxide concentrations, another straight line is obtained, as shown in Fig. 7. In terms of symbols,

$$\frac{d \cdot \frac{L}{D}}{dt} = K_2 c^n,$$

where n is again very nearly -3 (-3.11 as calculated).

The fact that the rate of solution of the changed lignin decreases so rapidly with increase in sodium hydroxide con-

It may now be of value to recapitulate and extend the conclusions drawn from the experimental results.

(1) With increasing concentration of sodium hydroxide the total delignification is increased.

(2) By the cooking of wood the lignin is obtained in a "colloidally soluble" form, but increase in the concentration of sodium hydroxide retards the dispersion of this lignin.

(3) This "colloidal" form of lignin is gradually acted upon by the sodium hydroxide, giving micellae which are more easily dispersed.

(4) The changed lignin in wood cooked with strong alkali cannot be peptized in pure water. It can, however, be peptized successfully in solutions of sodium hydroxide. Subsequent acidification and heating of the solutions result in reprecipitation of the lignin.

(5) The micellae of these sols may not consist altogether of unchanged lignin. They may represent a more or less hydrated derivative. The fact that part of the lignin is definitely changed in the cooking process is brought out by the difference generally found between the total lignin estimated after cooking and the lignin content of the original wood.

Colloidal lignin is a substance, presumably, of the lyophilic type. General information on lyophilic colloids may be found in text books on colloid chemistry, such as that of Freundlich (2). On the basis of such information, the following generalizations can be made: In salting out, lyophilic substances separate at high concentrations of alkali salts (several times molar). With alkalies such as sodium hydroxide, separation occurs only at very high concentrations. The substances come out generally in the form of amorphous flakes. The process is reversible; by dilution of the solution the separated matter can be redissolved.

In the present instance the behavior of the lignin is certainly similar to this. Thus the higher the concentration of sodium hydroxide the more difficult it is for the lignin to be dispersed. This effect is the opposite of salting out. The

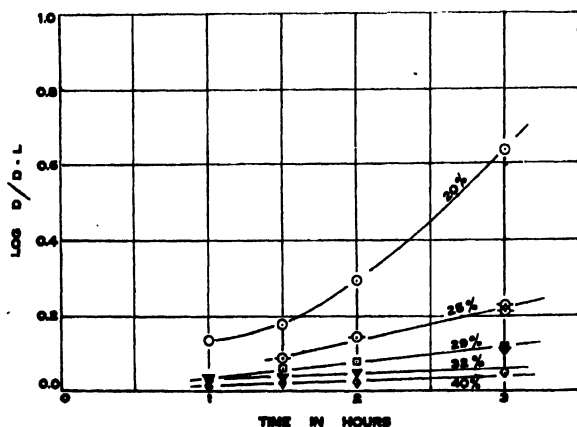


FIG. 8. Graph showing the relation between the quantity $\log D/(D-L)$ and the time of cooking, for different concentrations of sodium hydroxide.

fact that the lignin continues to go slowly into solution, even at the highest concentrations of sodium hydroxide, is to be interpreted as due to the continued breaking up of the larger micellae. Thus although most of the changed lignin may be in the form of micellae for which the sodium hydroxide concentration is sufficiently high to produce complete salting out, these micellae may be gradually broken up into smaller particles for which the alkali solution does not exert a salting out action.

If the logarithms of the ratios, $D/(D-L)$, be plotted against cooking times, straight lines are obtained for all concentrations of sodium hydroxide except the lowest (20%), as shown in Fig. 8. This indicates possibly the critical nature of this concentration, since below it the solution of lignin proceeds hand in hand with total cooking.

Throughout this discussion no reference has been made to the chemical nature of lignin or to the chemical reactions involved in cooking. The present work represents an attempt to follow lignin, considered in a general way, through different phases of a cooking process.

Acknowledgment

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To Dr. J. H. Ross the authors are greatly indebted for many valuable suggestions and for the interest he showed during the whole course of the research.

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PREPARATION OF THE ETHERS OF VINYL ALCOHOL¹

BY WILLIAM CHALMERS²

Abstract

The simple vinyl alkyl ethers are readily prepared from the β -bromo-ethyl ethers by the action of solid sodium hydroxide. Besides the vinyl ethers, the dialkyl ethers of diethylene glycol are formed in these reactions. β -Bromoethers are obtainable from the mono-ethers of ethylene glycol through the use of phosphorus tribromide. The constants of several vinyl ethers are given for the first time. The preparation of vinyl methyl ether, a gas at room temperature, and of vinyl *n*-butyl ether, b.p. 93.3° C., is described.

The following paper describes the results of a search for a convenient laboratory preparation of the vinyl alkyl ethers. The method developed proved suitable for the obtaining of desirable quantities of vinyl ethyl and butyl ethers. It led also to the preparation of vinyl methyl ether, although in small yield. The β -chloro-ethyl and phenyl ethers were prepared for the purpose of comparison.

Vinyl ethyl ether was first described by Wislicenus (17) who made it the object of an extensive study. At the time the present investigation was commenced the only reference to the existence of any other alkyl ether of vinyl alcohol lay in a patent specification (13)*. This did not give even the boiling points of the ethers quoted as examples (propyl and isopropyl). Besides the ethyl ether, divinyl ether (see 8) and two aromatic ethers (14) had been mentioned in the literature but on these also information was very scanty.

Since the discovery of vinyl ethyl ether there has been no dearth of announcements of new reactions in which it is formed. Wislicenus' original procedure was too laborious to be practicable. The one usually described in the standard textbooks has been developed by Claisen (3) and depends upon the possibility of extracting alcohol from diethyl acetal, $\text{CH}_3\text{CH}(\text{OC}_2\text{H}_5)_2$, to give the unsaturated ether. Phosphorus pentoxide, in the presence of quinoline, is used for this purpose. The method is rather unsatisfactory, not only on account of the low yield, but also because of the difficulty encountered in effecting a complete separation of the vinyl ether and unchanged acetal.

The addition of alcohols to acetylene, in a manner analogous to the commercial preparation of the vinyl esters, would seem to be the most direct manner of preparing the vinyl ethers. Nevertheless, it appears from the patent literature that, by substitution of alcohol for the acids in the process, the product is almost entirely acetal (4).

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*Abstracts of two new patent-processes have appeared since the present work was completed. One of these depends upon the reaction of the vinyl halides with the alcoholates of the alkalis and alkaline earth metals (9, 10). Another utilizes the catalytic action of metals of the first or eighth group in decomposing the acetals (11).

Vinyl phenyl and *p*-tolyl ethers have been made by Powell and Adams (14, p. 648) by distillation of the readily obtainable phenyl and tolyl β -bromo-ethyl ethers over powdered sodium hydroxide.

If it were possible to eliminate a molecule of water from the molecule of a mono-alkyl ether of ethylene glycol by the hypothetical reaction: $\text{CH}_2\text{OHCH}_2\text{OR} - \text{H}_2\text{O} \longrightarrow \text{CH}_2\text{:CHOR}$, a convenient preparation of the vinyl ethers would be at hand. A number of attempts to bring about this change directly in the case of the ethyl ether were fruitless. The utilization of the (now very cheap) glycol mono-ethers is possible, however, by their conversion into the β -halogenated ethers. Alkyl β -bromo-ethyl ethers were found to be readily converted into the vinyl derivatives by treating with solid sodium hydroxide at their boiling points.

It was necessary to devote attention not only to the reaction of the halogenated ethers with alkalis but also to their preparation from the glycol ethers. The hydroxyl group present in these compounds cannot be esterified by means of concentrated halogen hydracid as in the case of the simple alcohols, owing to the great ease with which their alkoxyl group is hydrolyzed. Phosphorus trichloride had been employed for this purpose by Karvonen (12, p. 1269) but the use of the tribromide had not apparently been tried. In fact, the only method of preparing the simple β -bromo-ethyl ethers described in the literature seems to be an indirect and tedious one described by Karvonen (12, p. 1269). Study of the reaction of phosphorus tribromide with the available glycol mono-ethers indicated that; while the yield of bromo-ether obtained from the monomethyl glycol ether was too small to be practicable, the ethyl and butyl β -bromo-ethyl ethers can be suitably prepared by its use.

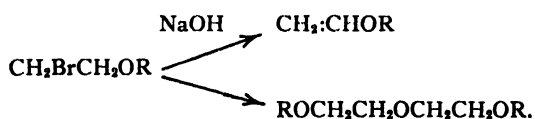
The preparation of the vinyl ethers is carried out as follows: A distilling apparatus with fractionating column is set up, the flask is partly filled with coarse, dry soda-lime as bearer and the bromo-ether and a large excess of powdered sodium hydroxide added. The flask is then immersed in an oil-bath which is heated moderately so that the temperature registered by a thermometer in the still-head does not rise appreciably above the boiling point of the vinyl ether. In this manner the low-boiling product is continuously separated from the bromo-ether which is retained in the reaction flask. After two to three hours nothing more comes over. The crude vinyl ether is separated from the water which has distilled over with it and treated with a convenient drying agent. There is usually a little unchanged bromo-compound present, but, owing to a considerable difference in the boiling points, one distillation through a good column is sufficient to remove it practically completely. The yields of vinyl ether obtained from the methyl, ethyl and *n*-butyl β -bromo-ethyl ethers are somewhat higher than one third of the theoretical.

As the β -chloro-ethers are much cheaper to prepare than the corresponding bromo-derivatives, a process which permits of their use would possess a decided advantage. The simple, mono-substituted compounds proved to be remarkably inert to the action of caustic alkali even at high temperatures. The di-substituted β,β' -dichloro-diethyl ether gave good yields of vinyl

β -chloro-ethyl ether with greater readiness. The possibility of decomposing this ether is to be ascribed to its elevated boiling point which provides a higher reaction temperature than in the case of the monohalogen compounds. For this reason, the reaction is found to proceed beyond the stage of the monovinyl ether with great slowness, divinyl ether being obtained only on long-continued heating*.

The low reactivity of the β -halogenated ethers, particularly the chloro-compounds, is clearly demonstrated by the severe treatment necessary to make them part with their halogen. The Finkelstein test (7, p. 1529) shows complete inertness of the substituted chlorine and bromine to sodium iodide. Quinoline, even after prolonged heating, produces no observable dehalogenation. While it might seem preferable to employ the alkali in solution, some experiments with dichloro-diethyl ether showed that the use of alcoholic potash leads to the production of high-boiling products, apparently ethoxy-derivatives.

The low yields of vinyl ethers obtained from the alkyl bromo-ethyl ethers are not due, as might be supposed, to an incomplete action of the dehalogenating agent. It is caused, in each case, by a side reaction giving a high-boiling, liquid by-product. These may be isolated if, after the formation of vinyl compound has ceased, the fractionating column be replaced by a short connecting tube leading to the condenser and the flask be strongly heated. For example, in the preparation of vinyl ethyl ether, a considerable quantity of a colorless liquid can be obtained which distils within a range of a few tenths of a degree of 186°C . These substances were found to be the dialkyl ethers of diethylene glycol. The amounts formed corresponded to the proportion of bromo-ether which had not been converted into the unsaturated ether, namely about 60%. Thus the reaction of alkyl β -bromo-ethyl ethers with sodium hydroxide takes place in two directions:



The formation of diethylene ethers is probably to be explained by the intermediate production of the sodium derivatives of the glycol mono-alkyl ethers. These would react further with unchanged bromo-ether to give the diethylene glycol ethers. Secondary products of this nature are not obtained from the less reactive dichloro-diethyl and phenyl β -bromo-ethyl ethers.

The vinyl ethers prepared, with the constants determined, are shown in Table I.

With the exception of vinyl methyl ether, which is a gas at room temperature, these compounds are mobile liquids. They have an odor resembling somewhat that of similar allyl derivatives. They may be kept indefinitely, even in bright sunlight, without change. A distinctive property which serves as a

*Hibbert and coworkers (8) have described the preparation of divinyl ether from dichloro-diethyl ether and noted the appearance of vinyl β -chloro-ethyl ether as an intermediate stage (8, p.1553).

TABLE I
CONSTANTS OF SOME VINYL ETHERS

Ether	Formula	Boiling point*, °C.	Density	Refractive index
Vinyl methyl	CH ₂ :CHOCH ₃	(gas) 12-14		
Vinyl ethyl	CH ₂ :CHOC ₂ H ₅	35.5	$\left\{ \begin{array}{l} D_4^{20} = 0.7589 \\ D_4^{9.2} = 0.7723 \end{array} \right.$	$n_D^{9.2} = 1.3856$
Vinyl <i>n</i> -butyl	CH ₂ :CHOC ₄ H ₉	93.3	$D_4^{20} = 0.7887$	$n_D^{20} = 1.4026$
Vinyl β -chloro-ethyl	CH ₂ :CHOCH ₂ CH ₂ Cl	108.0	$D_4^{20} = 1.044$	$n_D^{20} = 1.4362$
Vinyl phenyl	CH ₂ :CHOC ₆ H ₅	155.5	$D_4^{20} = 0.9776$	$n_D^{20} = 1.5226$

*The boiling points are all corrected.

convenient test is their almost explosive conversion into resinous polymeri forms on the addition of small amounts of iodine. A short study of these phenomena is described in the next paper (2).

Molecular Refractivities of the Vinyl Ethers

According to Eisenlohr (5), elements such as oxygen, which are capable of assuming a higher valency, cause anomalies in the refractivity when combined with another unsaturated group. Using the data given in Table I, calculation gives the molecular refractivities shown in Table II.

TABLE II
MOLECULAR REFRACTIVITIES OF VINYL ETHERS, CALCULATED FROM DATA IN TABLE I

Ether	M _D		Differences
	Found	Theoretical*	
Vinyl ethyl	21.89	21.85	+ .04
Vinyl <i>n</i> -butyl	30.94	31.08	- .14
Vinyl β -chloro-ethyl	26.65	26.71	- .06
Vinyl phenyl	37.49	36.72	+ .77

*Calculated from the atomic refractivities given by v. Auwers and Eisenlohr (6, p. 605).

It is seen that the aliphatic ethers give values for the optical constant which do not differ appreciably from the theoretical. Only in the case of the phenyl ether, where the aromatic radical forms an extension to the chain of conjugated unsaturated systems, is a small but distinct exaltation evident.

Experimental

Preparation of the β -halo-ethers

The new reactions carried out were as follows:

1. $\text{CH}_2\text{OHCH}_2\text{OCH}_3 \xrightarrow{\text{PBr}_3} \text{CH}_2\text{BrCH}_2\text{OCH}_3$ (B.p., 110° C.; yield, 10%)
2. $\text{CH}_2\text{OHCH}_2\text{OC}_2\text{H}_5 \xrightarrow{\text{PBr}_3} \text{CH}_2\text{BrCH}_2\text{OC}_2\text{H}_5$ (B.p., 127° C.; yield, 50-58%)
3. $\text{CH}_2\text{OHCH}_2\text{OC}_4\text{H}_9 \xrightarrow{\text{PBr}_3} \text{CH}_2\text{BrCH}_2\text{OC}_4\text{H}_9$ (B.p., 173-174° C.; yield, 37-43%)
4. $\text{CH}_2\text{OHCH}_2\text{OC}_6\text{H}_5 \xrightarrow{\text{SOCl}_2} \text{CH}_2\text{ClCH}_2\text{OC}_6\text{H}_5$ (B.p., 153-154° C.; yield, 50%)

Only the butyl compounds are new. The yields are calculated on the assumption that the reactions in each case are simple replacement of hydroxyl by halogen. They seem actually to be much more complicated.

The action of a large excess of hydrobromic acid (constant boiling point) upon glycol mono-ethyl ether was investigated. A vigorous reaction took place giving, on heating, a distillate of ethyl bromide with a small proportion of ethylene bromide (identified by boiling points and densities).

The preparation of the bromo-ethers is illustrated by the following example: *Butyl β-bromo-ethyl ether*. *n*-Butyl ether of ethylene glycol (810 gm., 7 moles), dimethyl aniline (300 cc., about 2 moles) and anhydrous ether (300 cc.) were placed in a two-litre, three-necked flask provided with a reflux condenser, stirrer and dropping funnel. The flask was cooled in ice and 200 cc. (slightly in excess of 2 moles) of phosphorus tribromide added dropwise with vigorous stirring. After the addition was completed, the mixture was heated on a water bath for several hours and allowed to stand overnight. It was then washed with much water and placed over potassium carbonate to dry. The solvent was separated by distillation, the crude bromo-ether coming over between 169-175° C. Yield, 427 gm. (37%). $D_4^{17} = 1.222$.

With smaller amounts a yield of 43% was obtained. The dimethyl aniline was added to remove the hydrogen bromide which was formed in considerable quantity.

Methyl and ethyl β-bromo-ethyl ethers were prepared in a manner quite similar except that, instead of tertiary amine, a large excess of the glycol ether was added. The reaction product in several cases was directly distilled without washing with water. When this is done, a thermometer must be inserted into the liquid itself and care taken that the temperature does not rise much above 240° C. Above 250° C. a violent decomposition sets in with the formation of free phosphorus.

Ethyl β-chloro-ethyl ether. This substance was prepared by allowing phosphorus trichloride to act upon glycol mono-ethyl ether. This preparation has already been carried out by Karvonen (12, p. 1269) who did not quote the yield he obtained. The writer succeeded in obtaining only 21% of the theoretical.

n-Butyl β-chloro-ethyl ether. This was prepared by the use of thionyl chloride, adopting a procedure similar to that employed by Bennett and Heathcoat (1) in making the ethyl ether. The yield given above was calculated on the basis of the equation:



Preparation of the Vinyl Ethers

It is not necessary to employ a copper flask in the preparation of the simple alkyl ethers as, under the conditions described, the alkaline mixture does not fuse.

The measurements of the densities of vinyl ethers were carried out by either a dilatometric or a direct-weighing (suspended-sinker) method. Except in the case of the ethyl ether, the indices of refraction were measured by means of an Abbé refractometer.

Vinyl methyl ether. From 26 gm. of methyl bromo-ethyl ether was obtained 4.5 gm. (approximately 40% yield) of the vinyl ether, following the general method described above. As the halogenated ether has a boiling point almost 100° C. higher than the vinyl ether, its separation offered no difficulty. The receiver was cooled in a freezing mixture and the material dried by sealing in a tube with anhydrous potassium carbonate. The boiling point was determined only approximately. A micro-gas analysis, made by Dr. E. W. R. Steacie, was unfortunately incomplete through an accident. It indicated, however, that the compound was quite pure: Calcd. for C_3H_6O ; vol. CO_2 /vol. H_2O = 1.50 or C/H = 0.500; found vol. CO_2 /vol. H_2O = 1.52 or C/H = 0.507.

The secondary product formed in this preparation, being very small in amount, was not examined.

On the analogy of Claisen's preparation for vinyl ethyl ether it might be expected that the action of phosphorus pentoxide on dimethyl acetal would lead to the formation of the methyl ether. An attempt to carry this out gave only a very small amount of a low-boiling liquid from which it was impracticable to attempt to separate any of the unsaturated ether formed.

Vinyl ethyl ether. Ethyl bromo-ethyl ether (400 gm.) was treated in three lots, the receiver being cooled with ice. After drying over potassium carbonate and fractionating, 83 cc. (63 gm., 33.5% yield) of pure ether of boiling point 35.4–35.6 ° C. was obtained.

The measurement of the refractivity of this compound was carried out on a goniometer using a hollow prism. The maximum attainable temperature was 9.2° C., owing to the rapid evaporation of the liquid.

The ethyl bromo-ethyl ether was refluxed for many hours with quinoline but no decomposition could be detected. Ethyl chloro-ethyl ether was refluxed for many hours with powdered sodium hydroxide, to which some sodium iodide had been added, but without appreciable decomposition of the ether. In order to elevate the temperature of the reaction, the same mixture was heated in an autoclave at 150° C. for several hours. Some vinyl ether was formed but only in very small yield. Another attempt was made by passing vaporized chloro-ether over soda-lime in a tube heated to 200–250° C., but again the yields were negligible. A small amount of a gas was generated. This was probably butadiene, as it is known that ethyl chloro-ethyl ether gives the diolefine when passed over soda-lime heated to a very high temperature (15, p. 49).

In attempting to dehydrate glycol mono-ethyl ether directly to the vinyl ether, several methods were tried. One of these consisted of distilling the glycol derivative over phosphorus pentoxide in the presence of an excess of carefully purified quinoline; another, of long refluxing over anhydrous zinc chloride. By the use of phosphorus pentoxide, only a very little low-boiling material was obtained. When zinc chloride was employed, some acetaldehyde and alcohol were formed, but no trace of vinyl ether could be observed. Distillation of the glycol ether in the vapor phase at moderate temperatures over several types of alumina, as well as thoria, led to extensive decomposition into acetaldehyde and alcohol, but gave no vinyl ether.

Formation of diethyl ether of diethylene glycol. The by-product obtained as described above in the preparation of vinyl ethyl ether, had a density of $D_4^{20} = 0.907$. It was completely soluble in five or six volumes of cold water. From its aqueous solution it could be salted out by the addition of potassium carbonate. Calcd. for $C_8H_{18}O_3$: C, 59.23; H, 11.19%. Found: C, 59.13, 59.00; H, 11.06, 11.00%.

For comparison a product of definite constitution was prepared in the following manner. The sodium derivative of the mono-ethyl ether of diethylene glycol ("carbitol") was formed by adding the calculated amount of sodium metal to a benzene solution of this compound. On treating this product with ethyl iodide, reaction took place smoothly. After purification, the resulting liquid had the constants: b.p., 186°C .; D_4^{20} , 0.908. It resembled the other material in solubility, odor, and high viscosity.

Vinyl n-butyl ether. From 150 gm. of butyl bromo-ethyl ether, in two lots, 28.5 gm. (34% yield) of the vinyl ether was obtained. A considerable quantity of water came over and the temperature in the still-head remained practically constant at 80°C . The crude ether was dried over potassium carbonate and fractionated several times. Calcd. for $C_6H_{12}O$: C, 71.93; H, 12.09%. Found: C, 71.88; H, 12.02%.

n-Butyl β -chloro-ethyl ether was refluxed for six hours over powdered sodium hydroxide. After fractionating, about 7% of this compound was found to have been converted into the vinyl ether.

Secondary product in the preparation of butyl ether. This had a boiling point of $245\text{--}247^\circ \text{C}$. When heated with hydrobromic acid it gave a distillate which consisted largely of butyl bromide (b.p., 101°C .), but also contained a small proportion of a liquid boiling between $215\text{--}220^\circ \text{C}$. (within which range lies the boiling point of diethylene glycol monobutyl ether). Its odor resembles that of the diethyl compound but its solubility in water is much less. The analysis agreed with that of dibutyl ether of diethylene glycol. Calcd. for $C_{12}H_{26}O_3$: C, 66.00; H, 12.00%. Found: C, 65.89; H, 11.87%.

Vinyl β -chloro-ethyl ether. This compound was obtained by treating β , β' -dichloro-diethyl ether with powdered sodium hydroxide in the same manner as the bromo-ethers. By separation of the unchanged material and re-treating, a total yield of 70% was obtained. The temperature of the still-head was kept at $110\text{--}120^\circ \text{C}$., and the heating prolonged for three hours. A few cc. of divinyl ether was also obtained. Analysis:— Calcd. for C_4H_7OCl ; Cl, 33.3%. Found: Cl, 33.2%.

Measurements, made by Mr. H. Wyman, of the density of this compound at three temperatures gave, besides that quoted above: $D_4^{25} = 1.038$; $D_4^{30} = 1.032$.

β , β' -Dichloro-diethyl ether was refluxed with alcoholic potash containing sodium iodide for several hours. The deposition of potassium chloride indicated that reaction had taken place. The bulk of the reaction product was a liquid distilling between 180 and 210°C . A little material boiling over the range $75\text{--}115^\circ \text{C}$. was obtained, but could not be fractionated to give a pure vinyl ether.

Vinyl phenyl ether. The preparation from phenyl bromo-ethyl ether has been already described by Powell and Adams (14, p. 648). In the present preparation, a yield of 33% vinyl ether was obtained in one distillation.

Reactivity of the β -halogenated ethers. To a 0.5-cc. portion of each of the chloro- and bromo-ethers prepared was added several cc. of pure acetone and 10 cc. of a 15% solution of sodium iodide in acetone. In no case was a precipitate of sodium chloride noticed even after standing for several days.

Acknowledgment

This work was done in the course of an investigation of broader scope begun under the direction of Prof. G. S. Whitby, now of the National Research Laboratories, Ottawa. The writer wishes to thank Dr. C. F. H. Allen and other members of the staff of McGill University for their encouragement and advice.

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POLYMERIZATION OF VINYL ETHERS¹BY WILLIAM CHALMERS²

Abstract

A number of vinyl ethers investigated were found to undergo violent polymerization reactions on the addition of free iodine. With very small amounts of the catalyst, considerable differences in polymerizability are disclosed, the phenyl ether being least sensitive to its action. The alkyl ethers, but not the phenyl ether, are very slowly converted into polymeric forms under the influence of heat and ultra-violet light. The orders of polymerization of the products range from 18 to 82. The "mer" refractivities of some of the polymers have been determined and shown to agree with the theoretical values calculated on simple assumptions. A closed-ring chain structure, with the structural units arranged in regular order and joined by normal, single bonds, is proposed.

In a preceding paper (3) the preparation of several vinyl ethers of the general formula $\text{CH}_2\text{:CHOR}$ has been described. In the following is given an account of some observations on the polymerization of these compounds under the influence of iodine, by heat, and by ultra-violet radiation.

In attempting to form the iodine-addition product of vinyl ethyl ether, Wislicenus (15, p. 113) discovered a reaction of an unusual nature. Although he did not get the expected di-iodide, a "balsamous" material was formed in a violent reaction on the addition of the halogen. When a very small proportion of iodine was added this was, indeed, taken entirely into true chemical combination, but the substance proved to be essentially a polymer of the original ether.

All the vinyl ethers studied in the present work, with the exception of divinyl ether, react with great violence on the addition of a quantity of iodine in excess of 0.5 to 5%, depending on the ether. The black, pitch-like products formed are in general soluble in all of the common solvents with the exception of the alcohols. When the iodine is employed in smaller proportions and in chloroform solution, much smoother reactions take place and the materials obtained are practically colorless.

Reduction of the amount of catalyst brings to light a large variation in the polymerizabilities of the different vinyl ethers. Thus, with 0.2% iodine, vinyl ethyl and vinyl *n*-butyl ethers are practically completely polymerized within 24 hr. but, even after six days, vinyl β -chloro-ethyl ether is only slightly affected and vinyl phenyl ether is unchanged. Vinyl ethyl and butyl ethers decolorize the added iodine solution immediately, become distinctly warm to the hand and remain so for several hours. In the case of the chloro-ethyl ether, the brown coloration takes 10 min. to disappear and the elevation of temperature is only slight. The vinyl phenyl ether never takes up completely even this small proportion of halogen and does not appreciably alter in temperature.

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² Holder of scholarships under the National Research Council of Canada, 1927-1931, at McGill University, Montreal, Canada, and at the University of Freiburg, Germany.

Wislicenus (15, p. 113) states that only a limited quantity of vinyl ethyl ether is polymerized by a definite amount of iodine. At least one part of the catalyst to 200 parts of ether is necessary to give complete polymerization, according to his work. If its action on the ethyl and butyl ethers has such a limit, it must be much lower than the value he gives. One part of iodine (in solution) was found to effect the entire conversion of 1000 parts of vinyl ethyl ether into polymer. This change required three days for completion. Vinyl β -chloro-ethyl ether shows, however, a definite limiting value. A quantity of this ether which had been sealed in a tube for 10 months with $\frac{1}{208}$ part of iodine, still contained 16% of unchanged material. Under these conditions, then, only 175 parts of vinyl chloro-ethyl ether are polymerized by one part of catalyst.

The ready polymerizability of these ethers through the agency of a specific catalyst would lead us to inquire into their behavior under the influence of heat and light. If we were to adopt the same conditions of treatment that have been found sufficient in other well-studied groups of polymerizable compounds, such an inquiry would fail to detect any heat- or light-sensitivity. The period of heating must be extended to several months and exposure to a strong source of ultra-violet radiation must be carried on continuously for many days before a thermo- or photo-polymerization of the alkyl ethers becomes evident. Long-continued heating or irradiation by ultra-violet light is almost without action upon vinyl phenyl ether.

Vinyl chloro-ethyl ether showed exceptional behavior, as it was found to be completely converted into a polymeric form on heating for three days. It is probable that some hydrogen chloride is formed by partial decomposition of the ether and that this acts as a catalyst for the polymerization. The photo-polymerizability is practically the same as that of the butyl ether.

All the vinyl ethers prepared polymerize with explosive violence when treated with a trace of the volatile metallic halides. On the other hand, long contact with metallic sodium is without effect.

These ethers differ considerably from other groups of vinyl compounds in the nature of their polymerizability. Comparison with the closely related vinyl esters (4) shows that, in the absence of catalysts, the latter are much more readily photo- and thermo-polymerizable. It should be pointed out that while the phenyl group shares with acetyl, etc., the property of being more "negative" in character than the alkoxyl groups, it is precisely the aromatic ether which is least sensitive to heat or light. In connection with the agency of iodine in bringing about the polymerization of the vinyl ethers, it is of interest that *p*-vinyl anisol (13, p. 489) has also been found to be readily converted into a polymeric form on the addition of this catalyst. Sensitivity to iodine seems, therefore, to be a specific property conferred by the presence of an ether-oxygen atom which need not necessarily be in juxtaposition to the vinyl group.* The accelerative effect of the halogen is in striking contrast to its distinctly

*The polymerizations of unsymmetrical diphenyl ethylene (7) and cinnamylidene benzyl cyanide (12, p. 3403) have also been observed to be catalyzed by iodine but the action is much weaker and seems to be different in mechanism from that observed in the case of the ethers.

anti-catalytic action in the polymerization of other substances such as styrene*, the vinyl halides (1, p. 321; 8, p. 1533), and methacrylic ester†.

Nature and Constitution of the Polyvinyl Ethers

A closer study of the polymerization products of vinyl ethyl, butyl and chloro-ethyl ethers showed that those obtained by different agents did not differ greatly in physical nature. With the exception of the soft solid formed on heating vinyl chloro-ethyl ether, they are all sticky, pitch-like materials, flowing only very slowly at room temperature but becoming quite fluid on heating above 100° C. They have a faint but characteristic odor when warmed. Solution in chloroform and other solvents takes place very slowly, indicating their high molecular nature. Determination of the molecular weights by freezing-point lowering in benzene showed that the number of molecules of monomer combined in the polymer molecules varied from 18 to 82 in different cases.

It was found impossible to distil any appreciable portion of polyvinyl ethyl ether in a high vacuum. It is likely that the volatile portion observed by Wislicenus (15, p. 113) was simply unchanged monomer.

The densities and indices of refraction of most of these materials were measured. Considerable light is thrown on their internal structure by calculation of the "molecular" refractivities from these data‡. For this purpose it is not necessary that the molecular weight be known. What is actually dealt with is the refractivity of the structural unit or "mer"§.

In the process of polymerization the double bonds originally present in the monomer disappear and are replaced by single bonds between the "mers" in the polymeric chain. If the degree of polymerization be high, we need not consider the question of the disposal of the end valencies, as the differences between the refractivities of the various possible structures will be so small as to be negligible. The theoretical "mer" refractivity is simply the sum of the atomic refractivities of the constituent atoms. The experimental value can be calculated from the density and index of refraction of the polymer by substituting these and the molecular weight of the monomer in the Lorentz-Lorenz formula.

The "mer" refractivities of several polyvinyl ethers are given in Table I. The constants employed in these calculations are listed at the end of this paper. For comparison, the molecular refractivities of the monomers (taken from the preceding paper (3)), are included.

The "mer" refractivities of each of the two pairs of poly-ethers investigated agree satisfactorily with the theoretical. The fact that the polymers obtained

*See reference to A. Krakau (14, p. 1261).

†Observations on the polymerization of this ester, made in conjunction with Dr. G. S. Whitby of the National Research Laboratories, will be published later.

‡The idea of utilizing the molecular refractivities of high molecular substances to elucidate their constitution is not new. The molecular refractivity of rubber was used many years ago by Gladstone and Hibbert (6, p. 680) and since then in this laboratory (9) to penetrate the structure of the rubber molecule.

§This convenient term has been introduced by Carothers (2, p. 2552).

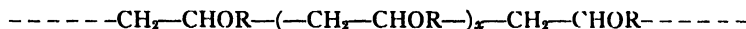
TABLE I
"MER" REFRACTIVITIES OF THE POLYVINYL ETHERS

Compound		Found	Theoretical*	Monomer (found)
Polyvinyl ethyl ether	(by heat)	20.14	20.11	21.89
	(by I)	20.38		
Polyvinyl <i>n</i> -butyl ether	(by I)	29.54	29.35	30.94
	(by light)	29.23		

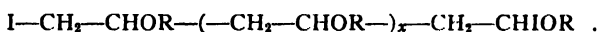
* Calculated from the atomic refractivities given by v. Auwers and Eisenlohr (5, p. 605).

by different methods vary somewhat in their densities and indices of refraction would not allow us to expect a more exact agreement. This is sufficiently close, however, to show very distinctly that in each polymerization the olefinic linkage has disappeared and has been replaced by two normal, unit bonds.

As polyvinyl ethyl ether gives oxalic acid on oxidation, it may be assumed that it and its analogues possess the regular structure:



Here, as in the case of most other high polymers, it is not easy to decide between an "open" or "ring" formula. In view of recent work on the polyoxymethylenes (11, p. 157), it might be considered that the iodine "polymers" are constituted entirely as the iodides:



It is interesting to note that Wislicenus (15, p. 113) long ago suggested that at least a portion of the "polymer" is made up of such a type of compound. This eminent worker further anticipated later views on the structure of macropolymeric bodies by his suggestion that the iodine is combined in a mixture of a series of "polymer-homologous"* compounds. The rest of the material he represented as a similar series of large rings formed by the separation of the iodine from the intermediately formed di-iodides. He did not decide between a regular or irregular arrangement of the structural units.

The di-iodide may possibly constitute an intermediate stage in the formation of the true polymer, but it cannot under ordinary conditions form more than a small proportion of the final product. The iodine polymer has an (average) order of polymerization of 82. Utilizing this fact, calculation shows that, when 0.1% of the iodine has been added, only 4.6% of the material can be present as a di-iodide.

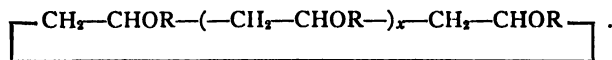
The bromine absorption of the polymers is very small but nevertheless measurable. This fact might be taken to prove the existence of an open-chain structure with an ethenoid linkage at one extremity, *viz.*:



*To employ an expression for this type of homology more recently suggested by Staudinger (10, p. 3025).

If the polymers possess this formula, the amount of bromine taken up should enable the number of "mers" per macromolecule to be reckoned. When this is done, the values obtained do not show the slightest connection with the orders of polymerization found by cryoscopic measurements. Hence the bromine absorption cannot be adduced in support of either this "open-chain" formula or that with free end valencies.

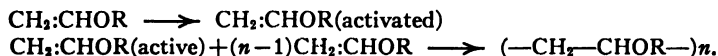
It is very likely that the polymers have actually a "closed-ring" structure:



The slight degree of unsaturation found is probably due to the separation of alcohol under the influence of the strong heating to which the materials are subjected during their preparation or purification. The analytical values obtained from all of the polymers are actually high in carbon and low in hydrogen, corresponding to such a loss.

Mechanism of the Polymerization of the Vinyl Ethers

In a later paper will be presented grounds for the assumption that, as in other typical polymerizations leading to molecules of considerable size, the reaction is to be considered as a "chain" reaction of the following type:



The first step, consisting in the formation of the "active" molecule, is believed to be unimolecular and to take place at a (comparatively slow) speed which determines the rate of the process as observed. The second step, wherein the formation of the polymeric molecule actually occurs, is completed at an exceedingly great rate. The writer considers it likely that, in the iodine polymerization, the "trigger" molecule is an addition compound with *one* atom of iodine and one free bond, *viz.*, $\text{ROCHI—CH}_2\text{—}$.

The course of the polymerization of the vinyl alkyl ethers under the influence of iodine is so easily controllable that it offers a tempting field for the study of the reaction velocities and, hence, of the mechanism of polymerization. The reaction seems to be free from autocatalysis and, from the above assumptions, should be (quasi) unimolecular.

Experimental

Polymerization of the Vinyl Ethers by Iodine

The rates of polymerization of the vinyl ethers were determined by adding 1 cc. of chloroform solution, containing 2 gm. of iodine per 100 cc., to 10-gm. quantities of the ethers. The liquids were allowed to stand at room temperature for the period of time noted in each instance. The extent of the change was determined by weighing the residue obtained after subjecting the material to reduced pressure, first at room temperature and then at 200-250°C., for several hours. The results of these measurements are shown in Table II.

Owing to the scarcity of material, no further work was done on vinyl methyl ether beyond the establishment of its sensitivity to iodine. When iodine is

TABLE II
POLYMERIZATION OF VINYL ETHERS BY 0.2% IODINE

Ether	Time	Polymer formed, %
Vinyl ethyl ether	6 hr.	88.3
Vinyl ethyl ether	24 hr.	100.0 (approx.)
Vinyl <i>n</i> -butyl ether	24 hr.	100.0 (approx.)
Vinyl β -chloro-ethyl ether	6 days	27.7
Vinyl phenyl ether	6 days	2.4 (polymer ?)

added to divinyl ether, the characteristic reaction does not take place, but the halogen is slowly replaced by a white, insoluble solid. This change was not further studied but it is probable that the body is an iodide of divinyl ether.

Attempt to distil polyvinyl ethyl ether. A sample of vinyl ethyl ether was polymerized by the addition of $\frac{1}{1000}$ part of iodine in chloroform solution. After three days, low-boiling compounds were removed by steam distillation. A distillate of the chloroform with a trace of unchanged vinyl ether was obtained. The material was then subjected to the action of superheated steam at 200° C. for several hours. Only a very thin film of an oily liquid could be observed on the surface of the water distillate. On heating the polymer in a distilling flask at a pressure below 0.1 mm. and at a slowly increasing temperature, eventually at 280° C., a mere trace of "balsamous" substance was observed in the receiver.

On heating, the originally amber-colored material becomes black owing to the liberation of free iodine. Continued heating in a high vacuum eliminates the halogen and gives a light-colored material.

Purification and Examination of the Iodine Polymers

The polyvinyl ethers obtained with 0.2% iodine were freed from low-boiling impurities as well as most, if not all, of their iodine content by long heating *in vacuo* and retaining over paraffin wax under reduced pressure for several weeks. The densities were determined by a flotation method using small drops of the material, usually in a methanol-water mixture. The suspending medium was freed from dissolved air and the average of a large number of determinations taken. The values are considered to be accurate to one unit in the third place of decimals. The refractive indices could be measured directly on an Abbé refractometer owing to the partial fluidity of the polymers. The constants obtained are shown in Table VI.

Polyvinyl ethyl ether. This compound, formed by the agency of iodine, has been analyzed by Wislicenus (15, p. 113) and shown to be (not considering the iodine content) a true polymer. Molecular weight measurements by freezing-point lowering in benzene gave: 5829, 5935.

The bromine absorption was determined with the usual precautions using a *N*/4 solution of bromine in carbon tetrachloride. Bromine solution was added to a weighed quantity of the polymer dissolved in chloroform until a light brown tinge of free bromine was apparent. The containing flask was then stoppered and placed aside for eight hours, when potassium iodide solution

was added and the iodine liberated titrated with standard thiosulphate solution. Potassium iodate solution was then added to determine if any formation of hydrogen bromide (substitution) had taken place, but none could be detected: 0.1850 gm. of polymer absorbed 0.64 cc. of bromine solution. Theoretically, for one double bond per "mer", 20.6 cc. of solution would be needed.

The polymer readily goes into solution on warming with fuming nitric acid. After neutralization, addition of calcium chloride solution gives a voluminous white precipitate which qualitative tests show to be the oxalate.

Polyvinyl butyl ether. Analysis gave: Calcd. for $(C_8H_{12}O)_x$: C, 71.93; H, 12.09%. Found: C, 71.72; H, 11.91%. Molecular weight, cryoscopic in benzene: 3944, 4025. Bromine absorption: 0.2551 gm. polymer absorbed 0.19 cc. bromine solution ($N/4$). Theoretically for one double bond per "mer", 18.8 cc. would be needed.

Polyvinyl β -chloro-ethyl ether. Analysis for chlorine by the method of Piria and Schiff: Calcd. for $(C_4H_7OCl)_x$: Cl, 33.3%. Found, 33.0%. The density was determined only roughly, using an aqueous calcium chloride solution as flotation liquid.

Action of Heat on the Vinyl Ethers

The rates of thermo-polymerization, at 130° C., of the vinyl ethers studied are listed in Table III.

TABLE III
RATES OF THERMO-POLYMERIZATION OF VINYL ETHERS

Ether	Time, days	Polymer formed, %
Vinyl ethyl ether	60	46.5
Vinyl <i>n</i> -butyl ether	60	26.0
Vinyl chloro-ethyl ether	3	100.0 (approx.)
Vinyl phenyl ether	90	1.6 (polymer ?)

The vinyl β -chloro-ethyl ether heat product. This is a soft, black solid insoluble in alcohol and chloroform even after long standing with these solvents. Beyond the determination of its density it was not further studied.

Vinyl ethyl ether heat product. On heating to 220° C., the sample gave a liquid which, on redistillation, boiled over the range 60–100° C. It did not contain more than a trace of vinyl ethyl ether but seemed to be composed largely of paraldehyde, as it had the characteristic odor and peculiar water-solubility (less soluble in hot water than in cold) of this compound. On retaining for a month *in vacuo* it lost weight. The composition was as follows: (1) liquid, 60–100° C. (43.5%); (2) loss of weight *in vacuo* (10%); (3) residue (polymer).

The residue was examined as follows: Analysis: Calcd. for $(C_4H_8O)_x$: C, 66.61; H, 11.19%. Found: C, 67.38, 67.44; H, 10.48, 10.25%. Molecular weight, cryoscopic in benzene: 1281, 1261.

Vinyl butyl ether product. Distillation of the sample gave the following results: (1) liquid; b.p., 93–120° C. (24%); (2) liquid; b.p., 120–180° C. (50%);

(3) residue (polymer). The first fraction reacts violently with iodine and is thus unchanged ether. The nature of the second fraction could not be ascertained. The residue was examined as follows: Analysis: Calcd. for $(C_6H_{12}O)_x$: C, 71.93; H, 12.09%. Found: C, 72.13, 72.04; H, 11.23, 11.19%.

Vinyl phenyl ether. After heating, came over almost completely at 155–157° C., indicating little or no change. A very small amount of an oily residue, insoluble in sodium hydroxide solution (and which, therefore, could not be phenol), was left in the flask. The material was not analyzed and whether or not it is a true polymer is doubtful.

Photo-polymerization of the Vinyl Ethers

Action of quartz lamp. Samples of vinyl butyl, chloro-ethyl, and phenyl ethers were exposed for seven days to the radiation from a quartz mercury-vapor lamp (110 volts, 3.5 amp.) in $\frac{3}{4}$ -in. tubes at a distance of 3 in. from the burner. Both the chloro-ethyl and phenyl ethers gave thin coatings of yellow, gelatinous solids on the walls of the tubes. The bulk of the product from the chloro-ether was, however, a material resembling the iodine polymer. The constants of this body were not determined owing to its lack of homogeneity. The phenyl ether showed no further change beyond the formation of the trace of insoluble material. The results obtained are listed in Table IV.

TABLE IV

EFFECT ON VINYL ETHERS OF IRRADIATION WITH A QUARTZ MERCURY-VAPOR LAMP (7 DAYS)

Ether Polymer formed, %	Vinyl <i>n</i> -butyl 61	Vinyl chloro-ethyl 66	Vinyl phenyl Trace (polymer ?)
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The photo-polymer of vinyl butyl ether was purified by retaining *in vacuo* over paraffin wax for several weeks. Bromine absorption: 0.4672 gm. polymer absorbed 0.22 cc. of bromine solution (*N*/4). Theoretically, for one double bond per "mer", 37.4 cc. would be needed.

Action of sunlight. Samples of all of the ethers prepared, including divinyl ether, were exposed in quartz as well as glass tubes, on a sunny window-ledge for periods extending from a month to a year. In no case was any observable change found on distillation.

Order of Polymerization and Bromine-absorption Measurements

The orders of polymerization calculated from the molecular weights, and the corresponding values calculated from the measured bromine absorption, on the assumption of an open-chain formula are shown in Table V.

Action of Other Agents on the Vinyl Ethers

Stannic chloride and antimony pentachloride, added in the proportion of one drop of 20% solution in chloroform to several cc. of the ethers, gave violent almost explosive, reactions. Resinous products are formed as with iodine, but these are marred by an intense black discoloration.

Samples of vinyl ethyl, butyl, and phenyl ethers were allowed to stand for several months over sodium wire. On distillation, the boiling points of the

TABLE V
ORDERS OF POLYMERIZATION OF SOME POLYMERS OF VINYL ETHERS

	Polyvinyl ethyl ether		Polyvinyl butyl ether	
	By iodine	By heat	By iodine	By heat
Order of polymerization calculated from cryoscopic measurements	82	18	40	—
Number of "mers" per double bond calculated from bromine absorption	32	—	100	170

liquids were found to be unchanged and no residues were left. A sample of vinyl butyl ether treated with several crystals of metaphosphoric acid became discolored but gave no appreciable amount of polymer on long standing.

TABLE VI
DENSITIES AND INDICES OF REFRACTION OF THE POLYVINYL ETHERS

	Density	Refractive index
Polyvinyl ethyl ether		
Iodine polymer	D_4^{20} : .975	n_D^{20} : 1.4624
Heat polymer	D_4^{20} : 1.010	n_D^{20} : 1.4769
Polyvinyl <i>n</i> -butyl ether		
Iodine polymer	D_4^{20} : .933	n_D^{20} : 1.4629
Heat polymer	—	n_D^{20} : 1.4748
Light polymer	D_4^{25} : .947	n_D^{25} : 1.4652
Polyvinyl β -chloro-ethyl ether		
Iodine polymer	D_4^{20} : 1.25	n_D^{20} : 1.4967
Heat polymer	D_4^{20} : 1.210	Opaque

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLII. THE SYNTHESIS OF CELLULOSE *o*-CHLOROBENZYL ETHERS AND MECHANISM OF THEIR FORMATION¹

BY R. H. BALL² AND HAROLD HIBBERT³

Abstract

A method has been developed for the study of the specific effect of the factors involved in the synthesis of cellulose *o*-chlorobenzyl ethers, and applied to an investigation of the most important of the reaction variables. The usefulness of the data thus obtained in studying the mechanism of the reaction, and in supplying new information on the chemical properties of soda cellulose, has been pointed out. The latter application of the data has been made possible by the development of a method for the quantitative separation of the cellulose mono- and lower ethers from the higher cellulose ethers.

It has been shown that in the reaction between soda cellulose and *o*-chlorobenzyl chloride, the extent to which ether formation occurs is influenced to a marked degree by the temperature of the reaction and the alkali concentration used in the preparation of the soda cellulose. Increase in either, or both, of these factors results in increased ether formation, such increase taking place, however, in a somewhat complex manner. It has been found possible to interpret the irregularities in the effect of these two variables, namely, temperature and alkali concentration, in accordance with a kinetic conception of the reaction, which assumes the existence of an equilibrium mixture of mono-, sesqui-, and di-sodium celluloses in the soda cellulose employed. Evidence has been presented for the definite existence of a sesqui-*o*-chlorobenzyl ether of cellulose, $C_{12}H_{17}O_{10}(C_7H_5Cl)_3$.

The effect of temperature on *o*-chlorobenzyl ether formation has been studied at one definite alkali concentration (20 gm. of sodium hydroxide per 100 cc.), and the effect of concentration at one definite temperature (95° C.) Based on the results obtained, a final experiment was performed, which showed that the influence of each variable is specific, and that these factors can be altered in such a manner as to lead, qualitatively at least, to the synthesis of ethers of well-defined structure.

The limited experimental data obtained on the influence of the duration of "steeping" and "aging" of the soda cellulose appear to indicate that during these processes a combination of oxidation and depolymerization changes occur, leading to an increase in the solubility of the cellulose derivatives formed.

An initial equilibrium apparently exists between the primary sodium cellulose and the sesqui sodium cellulose, the formation of the latter being favored both by increasing alkali concentration and higher temperature. The shifting of this equilibrium, with increase in the reaction temperature and concentration of the alkaline steeping liquor, in the direction of the sesqui sodium cellulose is followed immediately by the establishment of a second equilibrium between the latter and disodium cellulose, as shown by the fact that the sesqui cellulose *o*-chlorobenzyl ether can be obtained free from the cellulose di-ether only over a very limited range of temperature and concentration.*

Introduction

The present data regarding the synthesis and structure of the ethers of cellulose exhibit many peculiarities which do not afford a simple interpretation based on the generally accepted theory that cellulose is a supermolecular

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* An investigation of this equilibrium at various temperatures is to be carried out. (H.H.)

product made up of building units containing hydroxyl groups in the ratio of one primary to two secondary groups. Instances are available in which the substitution of one, of two, or of all three of the cellulose hydroxyl groups (per C_6 unit) appears to be the limit of the etherification, the extent of the reaction depending presumably on the nature of the etherifying agent and on the reaction conditions employed. Neither of these two factors has received any systematic study. In this paper the authors have initiated such a study by an investigation of the effect of reaction conditions on the yield and nature of the ethers formed by the interaction of soda cellulose and *o*-chlorobenzyl chloride.

The literature provides little explanation of the peculiarities of cellulose ether formation, as shown by the following examples. The dialkyl sulphates and aqueous alkali, in a multiple-stage reaction, have been found to substitute all three of the cellulose hydroxyl groups (3, 4, 9, 10). On the other hand, triphenyl methyl chloride, with pyridine as the condensing agent, is able to react with only the primary hydroxyl group, forming the mono cellulose ether exclusively (8). The interaction of ethyl chloride with soda cellulose was found to yield cellulose triethyl ether (1), while allyl bromide and soda cellulose react to form the diallyl ether of cellulose (21, 24). The reaction of cellulose with chloracetic acid and sodium hydroxide solution was found by Sakurada (22) to yield only the mono cellulose ether, whereas Chowdhury (2), by using more concentrated alkaline solution, succeeded in obtaining the tri-ether. In the interaction of benzyl chloride with soda cellulose several ethers are stated to be formed. Gomberg and Buchler (7) presented evidence for the existence of hemi-, sesqui-, and di-benzyl ethers of cellulose, but their method of purification has been shown to be unsatisfactory by Nakashima (12), who demonstrated that the actual products of the reaction were cellulose mono- and di-benzyl ethers, the latter representing the limit of the substitution, a result also supported by the work of Okada (14).

A careful review of the methods and conditions used in the investigations quoted above provides no general explanation of the limitations in reactivity shown by the hydroxyl groups of cellulose in ether formation. In only two cases is a ready explanation fairly obvious. For example, in the reaction with triphenyl methyl chloride, the well-known specificity of this reactant for primary hydroxyl groups accounts for the result obtained, while with chloracetic acid the concentration of sodium hydroxide solution appears to be the controlling factor. The other examples which have been cited afford no general theory explanatory of their peculiarities, especially since most of the reaction conditions have been varied so widely as to make it impossible to draw conclusions as to their effect on the extent of etherification.

The work on benzyl ethers of cellulose quoted above merits some further attention, as it indicates the possible effect of alkali concentration on the degree of substitution obtained. Thus, while Gomberg and Buchler were undoubtedly incorrect in the characterization of their benzyl ethers, they claim to have obtained a cellulose hemi-benzyl ether from a one-stage reaction using 20%

sodium hydroxide solution, a sesqui-benzyl ether from a one-stage reaction using 30% sodium hydroxide solution, and a di-benzyl ether from a two-stage reaction using 30% sodium hydroxide solution. Nakashima claimed a quantitative yield of the di-benzyl ether by using 40 to 50% sodium hydroxide solution, and Okada likewise used 50% sodium hydroxide solution in preparing the di-ether. Thus, by correlating the data of these investigations, a general relation between alkali concentration and degree of etherification appears probable.

Other ethers of cellulose, still more closely related to those which are the subject of this paper, have been prepared by Niethammer and König (13) by the action of *p*-chlorobenzyl chloride on cellulose. No pure ethers of cellulose were isolated by these workers, and the degree of etherification obtained was usually less than that of the mono-ether, in spite of purification with cuprammonium hydroxide solution. Their research appears to have been undertaken for the purpose of studying the effect of the reaction conditions on the nature of the products formed, but so many conditions were varied, and in such a haphazard manner, that it is impossible to draw any conclusions from their work.

From this brief review it appears that the limited ability of the individual hydroxyl groups of cellulose to form ethers is controlled in some way by the reaction conditions. Thus there is evidence that the concentration of the alkaline solution used to prepare the soda cellulose may have an important bearing on this point. On the other hand, other reaction conditions, such as temperature, molecular proportions, "aging" of the soda cellulose, use of solvents, etc., may have an equally important influence on the extent to which cellulose ethers are formed. The effect of these variables has received even less attention than that of alkali concentration. The present investigation was undertaken to study systematically the effect of the reaction variables on the yield and nature of the cellulose ethers formed from the interaction of soda cellulose and *o*-chlorobenzyl chloride, in the hope that the data obtained would have some value as such, and would also throw some light on the chemical nature of soda cellulose.

The structure of soda cellulose has been the subject of a great deal of research. It has long been known that cellulose, when immersed in aqueous alkaline solution, absorbs alkali in such a manner as to establish an equilibrium between that taken up by the fibre and that remaining in the solution. Although very little is known about the effect of temperature on this equilibrium, the effect of alkali concentration has received considerable attention, and has given rise to various interpretations based on the chemical structure of soda cellulose.

There is general agreement by most workers regarding the shape of the curve representing the absorption of alkali by cellulose. When cellulose is immersed in an aqueous sodium hydroxide solution, the concentration of alkali in the supernatant liquid shows a considerable decrease as a result of the alkali taken up by the cellulose. This might be attributed to adsorption if the amount of alkali associated with the cellulose did not show such a definite and irregular

relation to the initial concentration of the alkaline solution. Thus the amount of alkali taken up by the cellulose increases more or less steadily as the alkali concentration is increased to 16 gm. of sodium hydroxide per 100 cc. of solution, when no further absorption is observed until a concentration of 24 gm. per 100 cc. is exceeded. This irregularity in the absorption curve has generally been attributed to the formation of a definite chemical compound between cellulose and sodium hydroxide.

The quantitative study of the nature of this compound between cellulose and sodium hydroxide presents certain difficulties, and there has been considerable controversy as to whether it is a hemisodium cellulosate or a monosodium cellulosate. Of the two older methods for studying this problem, the "indirect" method was thoroughly investigated by Rassow (17), and the "direct" method by Heuser and Niethammer (11) and also by Rassow (17). The results of both of these methods point to the existence of a compound containing two glucose units of cellulose combined with one molecule of sodium hydroxide. Both methods, however, are open to serious criticism, as pointed out by Schwartzkopf (23), and too much confidence should not be placed in these results. A new method of differentiating between free and combined alkali has recently been developed by Percival, Cuthbertson and Hibbert (15). The data of these workers point very definitely to the fact that the break in the cellulose-sodium hydroxide absorption curve represents the formation of the compound $(C_6H_{10}O_5)NaOH$, containing 25.3 gm. of sodium hydroxide per 100 gm. of cellulose, and not $(C_6H_{10}O_5)_2NaOH$.

A clear picture of the significance of the cellulose-sodium hydroxide absorption curve is important for the interpretation of the data presented in this paper. The first portion of the curve represents the equilibrium between monosodium cellulosate and aqueous sodium hydroxide, the equilibrium favoring the former as the concentration of the latter is increased, until at 16 gm. of sodium hydroxide per 100 cc. the cellulose has been completely transformed into monosodium cellulosate. The break in the curve at this point further indicates that one hydroxyl group in each glucose unit of the cellulose is much more reactive towards alkali than the other two, since this hydroxyl group shows complete combination at a concentration considerably below that required for the other two to enter into reaction. This interval between the completion of the one reaction and the commencement of the other is responsible for the horizontal portion of the curve. The assumption that the first hydroxyl group to combine with sodium hydroxide is the primary one is based on analogy with the action of sodium ethylate on glycerol, by which α -monosodium glyceroxide is obtained exclusively (6). Furthermore, the continued rise in the curve after the concentration of 24 gm. of sodium hydroxide per 100 cc. has been reached indicates that the secondary hydroxyl groups enter into reaction with the sodium hydroxide above this point. Thus it is possible to draw a fairly clear picture of the effect of alkaline concentration on the reaction between cellulose and sodium hydroxide.

In the brief review of cellulose ether formation, it was pointed out that the specific ability of the individual cellulose hydroxyl groups to form ethers appeared to be controlled in some way by the reaction conditions. Since the reactants with which this study is concerned consist of *o*-chlorobenzyl chloride and the various sodium celluloses comprising "soda cellulose", the effect of the reaction conditions must be due chiefly to their influence on the amount and chemical nature of these sodium celluloses. Unfortunately it is impossible to apply the present information concerning soda cellulose directly to its reaction with alkyl halides, since this reaction usually requires a much higher temperature than has been used in the above investigations on soda cellulose (20 to 25° C.). Moreover, no studies have been carried out on the effect of temperature on the absorption of sodium hydroxide by cellulose. However, it seems probable that there will be some similar difference in the reactivity of the primary and secondary hydroxyl groups at higher temperatures, and that this difference will make itself evident in the cellulose ether reaction products.

Discussion of Results

The experimental results show the effect of time, temperature, concentration of sodium hydroxide solution, and time of steeping, on the products of the reaction between soda cellulose and *o*-chlorobenzyl chloride, and have been interpreted on the basis of the equilibrium between the different sodium celluloses which was postulated in the introduction. The general trend of these reaction variables has been determined, and sufficient experimental data obtained to enable conclusions to be drawn as to the mechanism of the reaction, and to indicate the possibilities of obtaining information from a further study of this subject.

The chlorine content of the cellulose *o*-chlorobenzyl ethers was used to determine the extent of ether formation. To facilitate the discussion of the subject, the calculated percentage of chlorine of each of the possible cellulose *o*-chlorobenzyl ethers is given below:

Cellulose hemi- <i>o</i> -chlorobenzyl ether $C_{12}H_{19}O_{10}(C_7H_6Cl)$	Contains one <i>o</i> -chlorobenzyl ether unit per two anhydro glucose units. (Insoluble)*	7.90% Cl
Cellulose mono- <i>o</i> -chlorobenzyl ether $C_{12}H_{18}O_{10}(C_7H_6Cl)_2$ or $C_6H_9O_6(C_7H_6Cl)$	Contains one <i>o</i> -chlorobenzyl ether unit per one anhydro glucose unit. (Insoluble)*	12.37% Cl
Cellulose sesqui- <i>o</i> -chlorobenzyl ether $C_{12}H_{17}O_{10}(C_7H_6Cl)_3$	Contains three <i>o</i> -chlorobenzyl ether units per two anhydro glucose units. (Soluble)*	15.25% Cl
Cellulose di- <i>o</i> -chlorobenzyl ether $C_{12}H_{16}O_{10}(C_7H_6Cl)_4$ or $C_6H_8O_6(C_7H_6Cl)_2$	Contains two <i>o</i> -chlorobenzyl ether units per one anhydro glucose unit. (Soluble)*	17.25% Cl
Cellulose tri- <i>o</i> -chlorobenzyl ether $C_6H_7O_6(C_7H_6Cl)_3$	Contains three <i>o</i> -chlorobenzyl ether units per one anhydro glucose unit. (Soluble)*	19.85% Cl

*Chloroform employed as solvent.

SOLUBILITY

The solubility of the reaction products was an important factor in determining the type of ether obtained from each reaction. Throughout all these experiments, with the exception of two in which the soda cellulose was aged for 12 and 48 hr. respectively, the cellulose *o*-chlorobenzyl ethers were characterized sharply by their behavior towards chloroform. Treatment with this solvent permitted of a sharp division into two different types: (a) those containing less than 12.35% chlorine, which were insoluble in chloroform, and (b) those containing more than 15.26% chlorine, soluble in this solvent. With the two exceptions noted above, no ethers were found having a chlorine content between 12.35 and 15.26%. It is significant that these values correspond very closely with those calculated for the mono-ether (12.37% Cl) and the sesqui-ether (15.25% Cl) respectively. We can therefore say that ethers up to and including the mono-ether are insoluble in chloroform, while the sesqui and higher ethers are soluble. Moreover, both in the temperature series of experiments (Fig. 2, curve 4), and in the alkali concentration series (Fig. 3, curve 3), the sesqui-*o*-chlorobenzyl ether was formed under the minimum conditions permitting the formation of a soluble ether. Since the mono-ether is insoluble in chloroform, as shown by repeated extraction, and by the absence of ethers having a chlorine content between 12.35 and 15.26%, the individuality of this sesqui-ether must be assumed in order to account for the isolation of a product containing 15.26% chlorine.

RELATION BETWEEN REACTION CONDITIONS AND YIELD AND NATURE OF THE CELLULOSE *o*-CHLOROBENZYL ETHERS FORMED(a) *Effect of Excess Alkaline Solution*

Much greater etherification was effected when the excess of the sodium hydroxide steeping solution was removed from the soda cellulose before its reaction with *o*-chlorobenzyl chloride.

(b) *Effect of Inert Diluents*

The presence of inert diluents, such as benzol or toluol, was found to hinder the reaction between soda cellulose and *o*-chlorobenzyl chloride.

(c) *Effect of Reaction Time*

This series of experiments, the results of which are plotted in Fig. 1, was

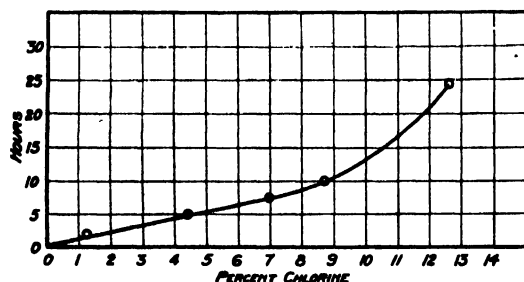


FIG. 1. Time curve. Alkali concentration, 20 gm. of sodium hydroxide per 100 cc. Temperature, 90°C.

carried out to determine a reasonable reaction time, and to test the reliability of the experimental procedure. The method of purifying the reaction products of this series resulted in a complete recovery of the ethers, both soluble and insoluble, but not in a separation into soluble and insoluble fractions.

Since a large number of experiments were to be performed, it was

necessary to arrive at a compromise between a reasonable degree of etherification and time of reaction. The times recorded in the literature vary from 2 (5) to 100 hr. (13). As the time curve found showed an almost linear relation between percentage of chlorine and time up to nine hours, and the reaction began to slow up beyond that point, ten hours was selected as a reasonable duration for the reaction.

The reliability of the methods used in carrying out these reactions and the purifying of the products is shown by the fact that the chlorine values in this series fall on a smooth curve. Each point on the curve represents a different experiment, carried out in exactly the same way as the other experiments in the series, except for an alteration in the length of time the reaction mixture was heated.

(d) *Effect of Reaction Temperature (Fig. 2)*

The results of the series of experiments dealing with the effect of temperature variation on the reaction between soda cellulose and *o*-chlorobenzyl chloride have been plotted in Fig. 2, curves Nos. 1 to 5. From these curves it can be seen that the extent of ether formation, as indicated by the yield and chlorine content of the products, increases in a complex way as the temperature is raised.

In Fig. 2, curve No. 1 has been plotted from a preliminary temperature study, and curve No. 2 from the final work on temperature. Reference to the Experimental Part will show that the series of experiments plotted in curve No. 1 (excluding the dotted portion) is similar to that summarized in curve No. 2, except that the reaction time was seven hours in the former series, and ten hours in the latter. Comparison between these two curves shows that there is the expected increase in the rate of ether formation with rise in temperature, but that the rate of this increase is suddenly diminished at 90 to 95° C. Since this slowing up occurs at the same temperature but at different percentages of chlorine in the two curves, it must be a phenomenon associated with that particular temperature, and not with the formation to completion of any specific ether.

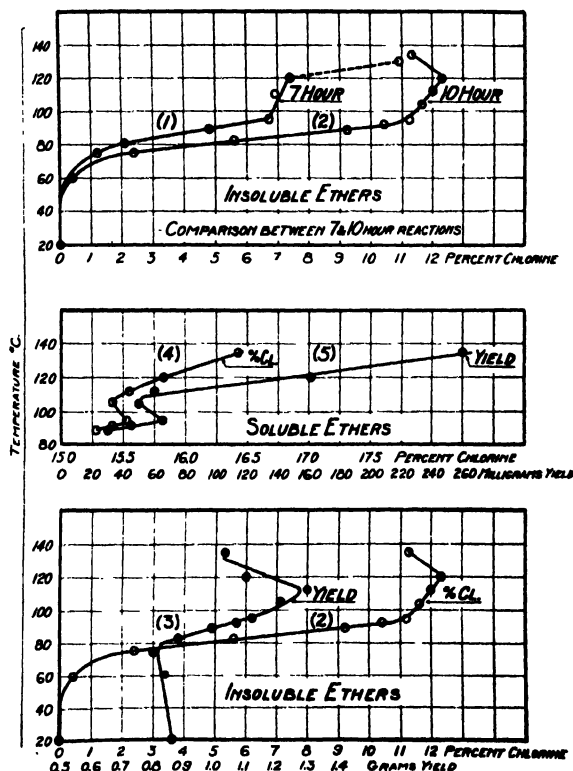


FIG. 2. Temperature curve. Alkali concentration, 19.9 gm. of sodium hydroxide per 100 cc. Time (except curve No. 1), 10 hr.

This is the only important point arising from a comparison of curves Nos. 1 and 2.

With this point in mind, the following conclusions regarding the effect of temperature on *insoluble* ether formation may be drawn from the shape of curves Nos. 2 and 3. As the reaction temperature is raised the rate of formation of the insoluble *o*-chlorobenzyl ethers, as measured by yield and chlorine content, increases until a temperature of 90 to 95° C. is reached. At this point the rate of increase slows up quite sharply, the insoluble ether formation finally passing through a maximum at 110 to 120° C. At the maximum point the insoluble product analyzed 12.35% chlorine, corresponding to cellulose mono-*o*-chlorobenzyl ether.

Turning to the effect of temperature on *soluble* ether formation, as presented in curves Nos. 4 and 5, it is seen that the soluble ethers make their appearance at about 90° C. with the formation of the cellulose sesqui-*o*-chlorobenzyl ether, and increase both in yield and in chlorine value as the temperature is raised, this increase taking place most rapidly after 110° C. has been exceeded. Thus the soluble ethers are first obtained at the temperature at which insoluble ether formation begins to slow up, and start to form rapidly at the temperature at which insoluble ether formation reaches its maximum. The most highly substituted soluble ether obtained in this series contained 16.42% Cl, considerably below that calculated for the di-ether.

(e) *Effect of Alkali Concentration (Fig. 3)*

The results obtained from the study of the relation between the concentration of the alkaline steeping solution and cellulose ether formation have been

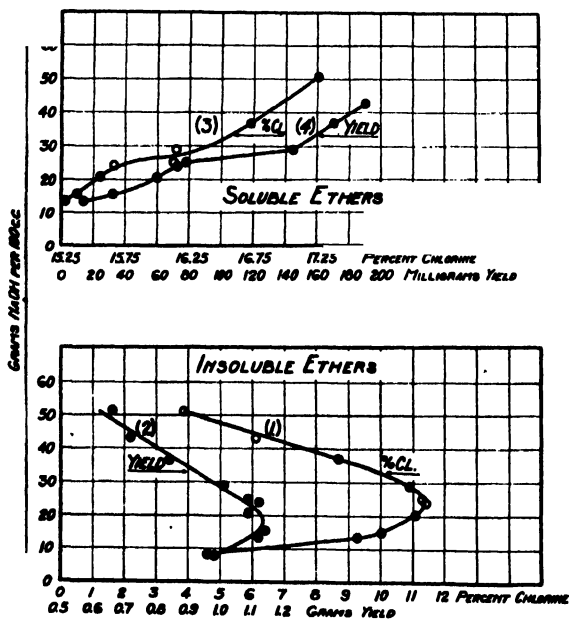


FIG. 3. Alkali concentration curve. Temperature, 95° C. Time, 10 hr.

plotted in Fig. 3. Here it is seen that there is an increase in ether formation as the concentration of the steeping solution is raised. The relations involved, however, are not simple. In this series of experiments the reactions were carried out at 95° C. for ten hours.

Curves Nos. 1 and 2 of this series show the effect of alkali concentration on the formation of the *insoluble* cellulose *o*-chlorobenzyl ethers. From these curves it is seen that, as the concentration of sodium hydroxide is increased to 25 gm. per 100 cc., the formation of the insoluble ethers increases to a maximum, giving

a product containing 11.38% Cl. Above this concentration both the yield and chlorine content of the insoluble ethers decrease rapidly.

Curves Nos. 3 and 4 of this series show the relation between alkali concentration and formation of *soluble* cellulose *o*-chlorobenzyl ethers. Soluble ethers begin to form at a sodium hydroxide concentration of 13.6 gm. per 100 cc., with a small yield of a product analyzing 15.27% Cl, corresponding to cellulose sesqui-*o*-chlorobenzyl ether. As the alkali concentration is raised, both the yield and chlorine content of the soluble ethers increase until a product corresponding to the di-ether is obtained at a concentration of 50 gm. of sodium hydroxide per 100 cc.

(f) *Effect of Altering the Time of Steeping*

This series of experiments is not extensive enough to require graphic presentation. Table V in the Experimental Part demonstrates that the yield of, and percentage of chlorine in, the soluble and insoluble ethers of cellulose decrease as the length of time of steeping is increased, maximum ether formation taking place with the shortest steeping time. This decrease is quite negligible in the case of the actual yield and chlorine content of the *insoluble* ethers, but is sufficient in considering the percentage of chlorine in the *soluble* ethers to call for further discussion, especially since two of the values obtained fall within the region 12.37 to 15.25% Cl, in which no products had previously been found. Thus the soluble *o*-chlorobenzyl ethers of cellulose resulting from the reactions using soda cellulose which had been steeped for 12 and 48 hr. respectively contained in both cases 14.14% Cl.

The phenomena associated with the aging of soda cellulose have been explained on the basis of oxidation, or depolymerization, or both (19, 25, 26). Either one of these processes would result in an increased solubility of the cellulose reaction product, and might conceivably render a part of the lower "insoluble" cellulose ethers soluble in chloroform. A decrease in the chlorine content of the soluble ethers, due to contamination by lower ethers rendered soluble through "aging" the soda cellulose, is, therefore, to be expected. This hypothesis is confirmed by the series of experiments under discussion, in which the decrease in the chlorine content of the soluble ethers is the only striking fact.

The formation from "aged" soda cellulose of a cellulose *o*-chlorobenzyl ether containing 14.14% Cl does not invalidate the arguments which have been presented for the existence of the cellulose sesqui-*o*-chlorobenzyl ether. The evidence for the existence of this ether has been based on two facts:— (a) the very specific percentage of chlorine which is shown by the soluble ether first formed in any series (15.27%); and (b) the insolubility in chloroform of the cellulose mono-*o*-chlorobenzyl ether, as shown by the fact that no *soluble* ethers were found containing less than 15.25% Cl, and no *insoluble* ethers analyzing more than 12.37% Cl. The only exceptions to these latter results are the two products containing 14.14% Cl. Since the peculiar solubility responsible for these products is associated with the changes taking place during long steeping, and this condition did not obtain in the rest of the work,

these two exceptions do not disturb the validity of the arguments presented for the existence of the cellulose sesqui-*o*-chlorobenzyl ether.

(g) Effect of Altering Reaction Temperature and Alkali Concentration Simultaneously

The data which have been presented on the effect of temperature and alkali concentration show that a high concentration of sodium hydroxide or a high temperature, individually, favor a high degree of substitution and a high yield of soluble cellulose *o*-chlorobenzyl ether. It therefore seemed probable that increasing the temperature and alkali concentration simultaneously would result in a high yield of highly substituted soluble ether.

This was confirmed in two experiments. In the first, using a reaction temperature of 120° C. and a sodium hydroxide concentration of 50 gm. per 100 cc., the yield of soluble ether from one gram of pulp was 1.02 gm., four times, that obtained in any of the other experiments performed, and the chlorine value of 16.85% was within 0.4% of that calculated for the di-ether. Both the yield and percentage of chlorine of the insoluble ether were very low. In the second experiment, using a reaction temperature of 120° C., a longer reaction time (15 hr.), and a sodium hydroxide concentration of 70 gm. per 100 cc., five grams of sulphite pulp yielded 6.6 gm. of soluble ether and only 0.65 gm. of insoluble ether.

MECHANISM OF THE REACTION BETWEEN SODA CELLULOSE AND
o-CHLOROBENZYL CHLORIDE

In interpreting the curves which have been presented in Figs. 2 and 3, one point especially must be kept in mind; namely, that unchanged cellulose has not been removed from the reaction products, and will be present in the fraction which has been called "insoluble ethers". This fraction, therefore, comprises cellulose mono-*o*-chlorobenzyl ether, lower cellulose ethers (if such exist), and unchanged cellulose. Since the reactions were run for a definite period, and not to completion, there will be a certain amount of unchanged cellulose resulting from most of the reactions. This cellulose could have been removed from the insoluble ether fraction by treatment with cuprammonium hydroxide solution, but it was found that four or five successive treatments would be necessary to effect this purification. The large number of experiments to be run made it necessary to omit the cuprammonium hydroxide treatment. Thus both the yield of, and percentage of chlorine in the insoluble ethers will be affected by the amount of unchanged cellulose present.

The most logical interpretation of the data presented in Figs. 2 and 3, and the one which finds greatest substantiation in our present knowledge of soda cellulose, is that based on the equilibrium between sodium hydroxide solution and the different sodium cellulosates. The experimental results obtained require the assumption that this equilibrium is influenced both by alkali concentration and by temperature. The former influence has been investigated at 20 to 25° C., but not at the temperatures necessary to form cellulose ethers. The influence of temperature on this equilibrium has not been studied hitherto.

(a) *Influence of Temperature on Soda Cellulose and its Reaction Products*

Consider first the effect of temperature increase on the *insoluble* cellulose ether formation, as presented in Fig. 2, curves Nos. 2 and 3, assuming that higher temperatures cause a shift in the equilibrium from the monosodium cellulosate towards the sesqui and disodium cellulosates. At the alkali concentration used in this series of experiments, namely, 19.9 gm. of sodium hydroxide per 100 cc., the monosodium cellulosate should be formed to completion at the lowest temperature used. As the temperature is raised, the rate of reaction between *o*-chlorobenzyl chloride and monosodium cellulosate will increase, resulting in (a) greater formation of cellulose mono-*o*-chlorobenzyl ether, (b) increase in the ratio of the latter to unchanged cellulose, and (c) a consequent increase in the chlorine content and yield of the insoluble ether fraction. This corresponds with the first portion of curve No. 2. The initial decrease in yield shown in the first portion of curve No. 3 is probably due to increasing solubility of cellulose in sodium hydroxide solution as the temperature is raised, an effect which is overtaken as the cellulose ethers begin to form rapidly. When a temperature of 90 to 95° C. has been reached, a shift in the equilibrium from mono to sesqui and disodium cellulosate commences, resulting in a reduction in the amount of monosodium cellulosate present in the reaction mixture. While this does not immediately cause a decrease in the chlorine content of the *insoluble* ether fraction, because of (a) the continued effect of temperature in increasing reaction velocity, and (b) the diminution in the amount of unchanged cellulose due to its transformation into soluble ethers, it is nevertheless sufficient to reduce the rate of increase of chlorine content which prevailed in the first portion of the curve. Finally, with further increase in temperature to 110 to 120° C., so much of the monosodium cellulosate has changed over to higher sodium cellulosates, and the amount of cellulose mono-ether formed has consequently become so reduced, that the ratio of cellulose mono-*o*-chlorobenzyl ether to cellulose commences to decrease, and the chlorine content of the insoluble ether fraction starts to drop. It should be pointed out that, due to decrease in the amount of unchanged cellulose, caused by its transformation into soluble ethers, the maximum in chlorine content of the insoluble ethers will come at a temperature above the true maximum, and that of the yield curve below the true maximum.

Curves Nos. 4 and 5, depicting *soluble* ether formation, confirm the above interpretation of the influence of temperature on this reaction. Thus the temperature of 90 to 95° C., at which the start of a shift from mono to sesqui and disodium cellulosates was postulated, is associated with the beginning of soluble ether formation. Furthermore, the temperature of 110 to 120° C. corresponds with a very rapid increase in soluble ether formation, supporting the assumption that a far-reaching change towards the higher sodium cellulosates has taken place by the time this temperature is reached, a change which is also responsible for the maximum value of insoluble ether formation.

The interpretation presented above suggests the following picture of the effect of temperature on a soda cellulose prepared from sodium hydroxide

solution containing 19.9 gm. of sodium hydroxide per 100 cc. The soda cellulose consists almost entirely of monosodium cellulosate up to a temperature of 90 to 95° C. At this point the formation of sesquisodium cellulosate, associated almost immediately with some disodium cellulosate, commences, thus reducing the amount of monosodium cellulosate. By the time the temperature has reached 110 to 120° C., this shift in the equilibrium is largely in favor of the higher sodium cellulosates.

(b) Influence of Alkali Concentration on Soda Cellulose and its Reaction Products

The interpretation of the curves in Fig. 3 is very similar to that which has just been presented for Fig. 2, except that it is not complicated by the effect of temperature on reaction velocity. The effect of alkali concentration on the sodium hydroxide-sodium cellulosate equilibrium at 20 to 25° C. has already been discussed in the introduction. Some similar relation may be expected at the temperature of 95° C. at which this series of reactions was conducted, although there is no reason to assume that the changes in equilibrium will come at the same concentration or be as clearly defined as at the lower temperature.

In the series of experiments studying alkali concentration, presented in Fig. 3, it is seen that the initial alkali concentration was 8.6 gm. of sodium hydroxide per 100 cc., which is probably below that required to cause complete formation of monosodium cellulosate at the temperature of the reaction. Therefore, as the concentration is increased, the amount of monosodium cellulosate in the reaction mixture will rise, bringing an increase in the chlorine content and yield of the insoluble cellulose ethers. This increase will tend to continue until the alkali concentration is sufficient to form the monosodium cellulosate to completion, unless, due to the temperature used, the higher sodium cellulosates start to form before the primary hydroxyl group has completely reacted. For the same reasons as were given in the discussion of the temperature curves, the formation of the higher sodium cellulosates, increasing as the alkali concentration is raised, will tend to lower first the yield, and then the chlorine content, of the insoluble ethers, resulting in a maximum in both these curves. The fact that the maximum in the percentage of chlorine of the insoluble ethers occurs at an alkaline concentration (23.9 gm. of sodium hydroxide per 100 cc.) above that necessary for soluble ether formation (13.6 gm. per 100 cc.) suggests that at 95°C. the formation of sesqui and disodium cellulosates commences at an alkali concentration below that necessary to cause complete formation of monosodium cellulosate.

From the discussion which has just been presented, it will be seen that the systematic investigation of the variables in the reaction between soda cellulose and an alkyl halide has provided a new angle from which to attack the problem of the chemical nature of soda cellulose, and has thrown some light on the constitution of this highly complex substance.

Experimental

(a) Method of Analysis

Since the chlorine atom in the *o*-chlorobenzyl group is stable to alkali under the conditions of cellulose ether formation, chlorine analysis was used to deter-

mine the degree of etherification of the cellulose *o*-chlorobenzyl ethers. In seeking a rapid and accurate method of chlorine analysis, numerous methods were tried, including the micro-method of Pregl (16, pp. 104-118), a micro-modification of the Carius method, the semi-micro method of Willard and Thompson (27), and the macro-method of Robertson (19, 20). The last three of these all proved reliable, with the Willard and Thompson method exhibiting several advantages over the micro-Carius for work on a small scale.

(b) *Preliminary Experiments*

In some of the work on cellulose ethers recorded in the literature, the excess of alkaline steeping solution was not removed from the soda cellulose before it was brought into reaction with the alkyl chloride, while other workers removed the steeping solution by pressure or suction. The relative merits of these two procedures were tested by two parallel experiments.

Experiment 1. One gram of cellulose* was steeped at room temperature for two hours with 10 cc. of 20% sodium hydroxide solution, 16 gm. of *o*-chlorobenzyl chloride added, and the reaction mixture heated for seven hours at 95° C. The reaction product was removed by filtration and purified by washing with alcohol and ether. Analysis:— Found: Cl, 0.19, 0.74%; mean, 0.43%.

Experiment 2. This was a duplicate of Experiment 1, except that the excess of alkaline solution was removed by pressure and suction before adding the *o*-chlorobenzyl chloride. Analysis:— Found: Cl, 6.64, 6.84%; mean, 6.74%. The improvement on removing the excess steeping solution is obvious.

Experiment 3. In the literature there are numerous references to the use of an inert solvent in the synthesis of cellulose ethers. The value of this procedure was tested by an experiment which duplicated Experiment 2, except that 15 cc. of toluene was added to the reaction mixture. Analysis:— Found: Cl, 3.13, 2.95%; mean, 3.04%. Comparison of this result with that of Experiment 2 shows that the use of the solvent is detrimental to cellulose ether formation.

Experiment 4. This duplicated Experiment 2 in every respect, and was run to see how closely results could be checked. Analysis:— Found: Cl, 6.56, 6.29%; mean, 6.42%.

Standard procedure. This work called for several series of experiments, to be run under conditions as nearly comparable as possible. As a result of the above experiments, the following was set up as a standard reaction procedure to be used throughout the work. One gram of cellulose was steeped in 10 cc. of sodium hydroxide solution of known concentration for a specified time in a 125-cc. Erlenmeyer flask closed with a rubber stopper. The wet soda cellulose was transferred to a sintered glass filter and placed under suction for two minutes, during which it was pressed down on the filter with a glass pestle. The same filter and water pump were used throughout the work. Suction was continued for only two minutes to minimize the formation of sodium

*The cellulose used in this investigation was highly purified alpha sulphite pulp provided by the Brown Company, Berlin, New Hampshire, to whom the authors wish to express their thanks for the gift of this material. It contained 96.4% alpha cellulose, and the entire quantity was dried in a vacuum drying oven to about 3% moisture content, prior to use.

carbonate. The soda cellulose was transferred from the filter to a 50-cc. round-bottomed flask fitted with a ground-glass condenser connection, 16 gm. of *o*-chlorobenzyl chloride added, and the mixture heated in an oil thermostat at the required temperature for a definite time interval. The purification of the reaction products was varied somewhat before a final procedure was arrived at, and will be described for each experimental series. Before analysis, the products were dried for 12 hr. at 80° C. and 20 mm. pressure in a slow stream of air drawn through a phosphorus pentoxide drying train. This drying procedure was found to be thoroughly efficient.

(c) *Reaction Time Series*

The procedure used in this series of experiments was standard, with conditions set as follows:— sodium hydroxide concentration, 20 gm. per 100 cc.; steeping time, two hours; reaction temperature, 90° C.; reaction time, variable. The products were purified in such a way as to recover both the soluble and insoluble ethers in one fraction. To effect this, a large excess of 95% alcohol was added to the reaction mixture to precipitate any cellulose ether which was dissolved in it. The products were then recovered by filtration, and washed thoroughly with alcohol, with water, again with alcohol, and finally with ether. The results of this series of experiments are summarized in Table I. The micro-Carius method was used in the analyses.

TABLE I
RELATION OF REACTION TIME TO TOTAL ETHERIFICATION

Time, hr.	2	5	7.5	10	24
Cl, %	1.40, 1.09	4.43, 4.47	7.08, 6.80	7.90, 9.57	12.57
Mean	1.25	4.45	6.94	8.73	12.57

(d) *Reaction Temperature Series*

Two series of experiments were carried out in the investigation of this variable, a preliminary and final series. They were similar except in reaction time and purification of the products. The preliminary series was run immediately after the four preliminary experiments, before the discovery of the solubility of the higher *o*-chlorobenzyl ethers of cellulose in the reaction mixture.

Preliminary series. The reaction procedure used was standard, with the conditions set as follows:— Sodium hydroxide concentration, 19.9 gm. per 100 cc.; steeping time, two hours; reaction temperature, variable; reaction time, seven hours. The purification procedure permitted of recovery of the insoluble ethers, contaminated by as much of the soluble ethers as was not removed by solubility in the reaction mixture. The reaction mixture, on cooling, was filtered, and the residue on the filter thoroughly washed with alcohol, water, alcohol, and ether, dried, and analyzed by the micro-Carius method. The results are presented in Table II.

TABLE II
EFFECT OF REACTION TEMPERATURE ON ETHERIFICATION (PRELIMINARY)

Temp., °C.	75	81	88	95	108	120	130
Cl, %	1.09	2.15	4.90	6.38	6.97	7.19	10.88
	1.29	2.12	4.68	6.84	6.89	7.59	11.04
Mean	1.19	2.13	4.79	6.74	6.93	7.39	10.96

That the above figures, with the exception of the values at 130° C., represent substantially the chlorine content of the insoluble cellulose ethers, can be shown from the data to be presented on the final temperature series in Table III. Reference to this table shows that up to 120° C. only 160 mg. of soluble ethers could be present in a total ether yield of about one gram. Part of these soluble ethers would be lost by their solubility in the reaction mixture. Therefore it may be concluded that, in the figures presented in Table II, and plotted in Fig. 2, curve No. 1, contamination of these insoluble products by soluble cellulose *o*-chlorobenzyl ethers could cause only a very slight increase in their chlorine content. This is not the case in the experiment carried out at 130° C., since a much greater amount of soluble ether is formed at this temperature, and the reaction mixture is more difficult to filter. However, it may be said that, with the exception of the value at 130° C., the figures presented in Table II and plotted in Fig. 2, curve No. 1, represent substantially the effect of reaction temperature on the formation of insoluble cellulose *o*-chlorobenzyl ethers during a seven-hour reaction.

Final temperature series. The reaction procedure used in this series of experiments was standard, with the conditions set as follows:— Sodium hydroxide concentration, 19.9 gm. per 100 cc.; steeping time, two hours; reaction temperature, variable; reaction time, 10 hr. The purification procedure permitted of a separation of the products into soluble and insoluble fractions in the following manner. The reaction mixture was filtered and the washing of the insoluble material carried out as described in the preliminary temperature series. The soluble ether remaining in this fibrous part was then removed by a three-hour continuous extraction with chloroform. The insoluble residue from this extraction was thoroughly washed with alcohol and ether, and given the usual drying, and constituted the *insoluble* cellulose *o*-chlorobenzyl ether fraction. To recover the dissolved cellulose ether, the filtrate from the reaction mixture was added to 150 cc. of petroleum ether (b.p., 30–50° C.) and allowed to stand for several hours, during which period the precipitated product coagulated and settled to the bottom of the flask. This precipitate was filtered off, washed with alcohol, and dissolved in the chloroform which was used in the extraction of the insoluble product. This chloroform solution, which now contained all the *soluble* cellulose *o*-chlorobenzyl ether, was dropped slowly into a flask containing rapidly stirred, boiling water. This removed the chloroform and precipitated the cellulose ether in granular form. The product from this precipitation was ground to a fine powder in an

agate mortar, thoroughly washed with alcohol, and given the usual drying. The results of the experiments in this final temperature series are shown in Table III.

TABLE III
EFFECT OF REACTION TEMPERATURE ON ETHERIFICATION (FINAL SERIES)

Temperature °C.	Insoluble ethers		Soluble ethers	
	Yield, gm.	% Cl*	Yield, mg.	% Cl**
20	0.86	0	0	
60.5	0.84	0.4	0	
75.5	0.80	2.41	0	
82.5	0.88	5.60	0	
89	0.99	9.19	30	15.27
92	1.07	10.47	45	15.41
95	1.12	11.18	65	15.52
105	1.21	11.67	50	15.41
112	1.30	12.00	60	15.55
120	1.10	12.35	161	15.82
135	1.03	11.27	260	16.42

Method of analysis:— *Robertson; **Willard and Thompson.

(e) *Alkali Concentration Series*

The reaction procedure used in this series of experiments was standard, with the conditions set as follows:— Sodium hydroxide concentration, variable; steeping time, two hours; reaction temperature, 95° C.; reaction time, 10 hr. The purification procedure was the same as that used in the final temperature series, this permitting of a separation of the products into soluble and insoluble ethers. The analysis of the products is given in Table IV.

TABLE IV
EFFECT OF ALKALI CONCENTRATION ON ETHERIFICATION

Concentration, gm. NaOH/100 cc.	Insoluble ethers		Soluble ethers	
	Yield, gm.	% Cl*	Yield, mg.	% Cl**
8.6	0.98	4.62	0	
13.6	1.12	9.31	13	15.27
15.6	1.14	10.05	32	15.38
20.6	1.09	11.09	60	15.55
23.9	1.12	11.38	72	15.66
25.2	1.09	11.30	78	16.12
29	1.01	10.89	145	16.14
36.9	0.84	8.71	170	16.72
43.1	0.72	6.07	190	15.26
50.8	0.66	3.87	150	17.26

Method of analysis:— *Robertson; **micro-Carius.

(f) *Series on Time of Steeping*

The reaction procedure used in this series of experiments was standard, with the conditions set as follows:— sodium hydroxide concentration, 18.9 gm. per 100 cc.; steeping time, variable; reaction temperature, 95° C.; reaction

time, 10 hr. The purification procedure was the same as that used in the final temperature series, permitting of a separation of the products into soluble and insoluble ethers. The analysis of the products is presented in Table V.

TABLE V
EFFECT OF TIME OF STEEPING ON ETHERIFICATION

Steeping time, hr.	Insoluble ethers		Soluble ethers	
	Yield, gm.	% Cl*	Yield, mg.	% Cl**
1/12	1.15	11.24	60	15.70
1/2	1.14	11.01	60	15.73
2	1.12	11.18	65	15.52
12	1.14	11.09	55	14.14
48	1.12	10.83	50	14.14

Method of analysis:— *Robertson; **Willard and Thompson.

(g) *Effect of Increasing Alkali Concentration and Reaction Temperature Simultaneously*

Two experiments were run to check this point.

(1) The reaction procedure used was standard, with the conditions set as follows:— sodium hydroxide concentration, 50 gm. per 100 cc.; steeping time, two hours; reaction temperature, 120° C.; reaction time, 10 hr. The purification procedure permitted of the separation of the products into soluble and insoluble ethers, but had to be modified from that previously used for this purpose because the fibrous structure of the product was destroyed to such an extent that it was impossible to filter the reaction product. The products were therefore purified in the following way. The reaction mixture was subjected to steam distillation for four hours to remove as much of the *o*-chlorobenzyl chloride as possible. The pasty product from this procedure was then given a four-hour extraction with chloroform in a Soxhlet apparatus. The insoluble residue, after the usual washing and drying, weighed 0.26 gm., and contained 4.53% chlorine.

The usual method of precipitating the soluble ethers from the chloroform solution gave a plastic product which could not be powdered or purified by washing. This was probably due to small amounts of *o*-chlorobenzyl chloride and *o*-chlorobenzyl alcohol which had not been removed by the steam distillation. The plastic product was therefore incorporated in a mortar with a large amount of crystalline sodium chloride, and given a four-hour extraction with alcohol in a Soxhlet apparatus. The salt was then removed from the extracted material by washing with water, and the soluble cellulose ether recovered as a powder with none of its previous plastic properties. The soluble *o*-chlorobenzyl ether of cellulose recovered from this reaction weighed 1.02 gm., and on analysis showed:— Cl; 16.97, 16.74%; mean, 16.85%.

(2) In the second of these experiments the reaction procedure used was standard, except that the quantities in each case were five times larger. The

reaction conditions were:—sodium hydroxide concentration, 70 gm. per 100 cc.; steeping time, 15 min.; reaction temperature, 120° C.; reaction time, 15 hr. The purification of the products was carried out in the same way as in the previous experiment except that the removal of impurities by incorporation with salt and alcohol extraction was carried out immediately after the steam distillation, and was then followed by the chloroform extraction. A yield of 0.65 gm. of insoluble product was obtained, showing the following analysis by the method of Robertson:—Cl, 9.17, 9.08%; mean, 9.12%. A yield of 6.6 gm. of soluble ether was obtained, giving the following analysis by the method of Willard and Thompson:—Cl: 16.60, 16.61%; mean, 16.60%.

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SOME CONSIDERATIONS IN REGARD TO EXPERIMENTS WITH CHEMICAL HERBICIDES¹

BY GEO. L. GODEL²

Abstract

The writer reviews the subject of weed control by chemicals in the growing crop mainly with reference to prairie conditions, and discusses it under the following heads: the herbicide, the sprayer, weather conditions, the crop, the experimental technique. A technique is described which permits the analysis of a large group of variable factors in the weed-control experiments carried on in Saskatchewan.

An extensive program of weed research was undertaken in Saskatchewan in 1929, to determine the practicability of both methods of control by chemicals (control of perennial weeds in small patches, and control of annual weeds in the crop) under semi-arid conditions and with extensive farming methods. The experiments were conducted under the most variable conditions of soil and climate, and many chemical solutions were tested at varying concentrations and amounts of spray per acre. Treatments were made on stinkweed and wild mustard in wheat, oats, barley, rye, corn, sweet clover, western rye grass, alfalfa and brome grass. During the last three years the effects of the following chemicals have been studied: sodium chlorate, sodium dichromate, copper nitrate, copper sulphate, sulphuric acid, iron sulphate, sodium chloride and ammonium bisulphate. All of these, except the last, reduced the weed infestation of the treated crops; the chemicals are listed in order of effectiveness. The results showed, however, that many factors other than proper strength of solution and amount of spray per acre should be taken into account.

The largest percentage of control of wild mustard in the crop of 1931 was obtained with weak solutions of sodium chlorate. The effects of this chemical on the crop are more severe than those of the others, and therefore its use should be further investigated before it can be recommended.

The application of dry chemicals for control of weeds in the crop combine in general two farming operations—fertilizing of the soil and weed control. These methods offer little promise for Saskatchewan as they require cool, foggy, wet weather which is rarely found in the west, and because it has been found that, in order to be effective here, commercial fertilizers have to be drilled in with the seed and not broadcasted on the surface.

It appears that, under Saskatchewan conditions, only a few weed species can be controlled successfully. In wheat, sulphuric acid will control common wild mustard, Indian mustard, wild radish, stinkweed, false flax, tumbling mustard and wild buckwheat. New shoots of Canada thistle are somewhat retarded in their development. Hare's ear mustard, ball mustard, cow cockle, lamb's-quarters, Russian pigweed, and the grasses, wild oats, darnel, etc., could not be controlled under the conditions of these experiments. It is thus important that chemical treatments be limited to fields where weed infestation consists mostly of susceptible weeds, because in controlling these the crop and resistant weeds are stimulated. At Watson, Sask., in 1931, when wild mustard or stinkweed was associated with either wild oats, lamb's-quarters or Russian pigweed, there was always an increase in the development of the latter weeds after treatment.

In these experiments the cereals ranked as follows in resistance to sulphuric acid sprays:—resistant; wheat, oats: intermediate; barley, spring rye: susceptible; corn.

Introduction

Weed control experimentation with herbicides is carried along two different lines; the control of perennial weeds in small patches, and the control of annual weeds in the crop. These two methods are absolutely distinct in that one aims at complete eradication of the root system of troublesome perennial weeds, it renders the soil barren for an indefinite period of time and it is expensive and therefore limited to the treatment of weeds in small patches in

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the field, or on land inaccessible to cultivation. The other method is intended for the control of certain annual weeds in a crop; it causes little injury to the latter and no harmful effect to the soil; it is cheap and intended for the treatment of large areas thoroughly weed-infested.

Annual weeds are troublesome in a field because they compete with the crop for mineral nutrients, space, light, and especially soil moisture, a limiting factor in crop production in western Canada, and thus occasion a great loss in yield of harvested farm products. Most annual weeds are probably still more troublesome because of their earliness which allows them to shatter their seeds in the field before harvest. Goss (30) has demonstrated that most weed seeds possess the ability to retain their viability over long periods of time, and a tendency to delayed germination.

Weed control by chemicals may become of considerable importance to prairie farmers for the control of wild mustard and stinkweed in a cereal crop because these weeds are extremely difficult to control by cultural methods.

The treatment of weeds in the crop fulfils two purposes: it favors the growth of the cereal because it almost eliminates weed competition; it also prevents weed seeds shattering before harvest and thus acts as a complement to good tillage, rendering possible in the course of time complete eradication of wild mustard and stinkweed on the farm.

Chemical control in the crop is a common practice on the larger farms of Europe where the method was first used; in America the method has been tried from time to time, but in spite of the recommendations of Bolley and others, it has never passed the experimental stage. In Saskatchewan, the Department of Agriculture undertook in 1929, in co-operation with the University of Saskatchewan, an extensive program of weed research to determine the practicability of both methods of control by chemicals under semi-arid conditions and with extensive farming methods. This work was later carried on in collaboration with the National Research Council of Canada and the Departments of Agriculture of the provinces of Alberta and Manitoba.

These experiments were conducted under the most variable conditions of soil and climate and many chemical solutions were tested at different concentrations and amounts of spray per acre. Treatments were made on stinkweed and wild mustard in wheat, oats, barley, rye, corn, sweet clover, western rye grass, alfalfa and brome grass.

The experiments have confirmed the author's deductions arrived at after an extensive survey of the literature. Both clearly indicated that weed control by chemical sprays in a growing crop is subject to the action of a large number of factors, the importance of which must be realized if successful control is to be obtained in all cases.

The writer has attempted to review the subject of weed control by chemicals in the growing crop, but mainly from the standpoint of prairie conditions. More experiments will be conducted in the next year or two but future trials will consist mostly of extensive field experiments to determine more accurately the cost of treatment per acre, and to study the relative value of the different types of spraying equipment.

The subject is discussed under the following heads: the herbicide, the sprayer, the weather conditions, the crop, the experimental technique.

The Herbicide

A search of the literature indicates that a large number of chemicals can be used for the control of annual weeds in a growing crop. The methods of application of the chemicals can be grouped into two classes: chemical sprays and chemical dusts, broadcasted like most commercial fertilizers.

The most common methods in use belong to the first group. These in turn can be divided into: (a) acid sprays, which act by burning the plant tissues; (b) chemical sprays which are toxic to plants.

The application of dry chemicals for the control of weeds in the crop is of fairly recent date, but the results which are reported from Europe are most interesting and might prove of some value in the more humid districts of the American continent. These methods combine in general two farming operations; fertilizing of the soil and weed control. They offer little promise for Saskatchewan because, first, they require weather conditions which are rarely found in the west (cool, foggy, rainy weather); and second, because it has been proved that in order to be effective here, commercial fertilizers have to be drilled in with the seed and not broadcasted on the surface.

These different methods cannot be fully discussed in the scope of one article. The reader is referred to reports by the following investigators for further information:

Chemical sprays:—sulphuric acid; Rabaté (71), Aslander (4, 5): copper sulphate; Long (47), Bolley (10): copper nitrate; Dusserre (25, 26): iron sulphate; Korsmo (45), Olive (62), Shutt (84): sodium chloride; Marre (53, 54): sodium chlorate; Loyer (50).

Dry chemicals:—powdered sodium chloride; Crépin (20): kainit; Vasters (89): potassium bisulphate; Fron (29): ammonium sulphate, sylvanite and cyanamide; Jaguenaud (38-41).

Other chemicals which have been tested include chiefly ammonium chlorate, sodium nitrate, sodium arsenite, sodium dichromate, ammonium bisulphate, and sodium hydroxide.

In Saskatchewan, the following chemicals have been studied during the past three years: sulphuric acid, copper sulphate, copper nitrate, iron sulphate, sodium chloride, sodium chlorate, sodium dichromate, and ammonium bisulphate. All these chemicals, with the exception of ammonium bisulphate, considerably reduced the weed infestation of the treated crops, but the results indicated clearly that many factors other than proper strength of the solution and right amount of spray per acre had to be taken into consideration. In order of effectiveness pound per pound, disregarding such factors as cost of application, solubility of the chemical, etc., the above-mentioned chemicals seemed to rank as follows, under Saskatchewan conditions: sodium chlorate, sodium dichromate, copper nitrate, copper sulphate, sulphuric acid, iron sulphate and sodium chloride. To give similar results, these chemicals would therefore have to be applied at different rates and for the control of common

wild mustard, the factor which will determine their usefulness from a practical standpoint, will be price per pound of pure chemical. It takes for instance about six times as much sodium chloride as copper sulphate, five times as much iron sulphate as copper nitrate, five times as much sulphuric acid as sodium chlorate (amounts by weight). The amounts of spray and strength of the solution shown in Table I would give, under average conditions in Saskatchewan, similar control of wild mustard in wheat.

TABLE I

CONCENTRATION AND QUANTITY OF SPRAY REQUIRED TO GIVE SIMILAR CONTROL OF WILD MUSTARD IN WHEAT UNDER AVERAGE CONDITIONS IN SASKATCHEWAN

Chemical	Concentration, %	Dosage per acre, gal.	Chemical	Concentration, %	Dosage per acre, gal.
Sodium chlorate	1½	75	Sulphuric acid	8	75
Sodium dichromate	3-3½	75	Iron sulphate	25	75
Copper nitrate	3½	75	Sodium chloride	25	125
Copper sulphate	5-5½	75			

The largest percentage of wild mustard control in the crop in 1931 was obtained with the application of weak solutions of sodium chlorate and such a treatment would have the great advantage of being the cheapest as far as cost of application is concerned, but the effect of this chemical solution on the crop is more severe than that of the other chemicals and the method has to be further investigated before it can be recommended.*

Sulphuric acid treatment seems to injure the sprayed crop to a considerable extent, because it burns and bleaches most of the leaves which the cereal had formed at time of treatment: if the treatment is done early, the damage disappears long before harvest. Because of its mode of action on the weeds, sulphuric acid permits the control in the crop of more weed species than is the case with the other chemicals mentioned.

The use of copper nitrate is much restricted because of the high cost of the chemical.

Sodium dichromate should be used with caution because it acts somewhat by burning the plant tissues and also by its great toxicity.

Copper sulphate sprays are fairly advantageous but their use is limited exclusively to wild mustard and closely related species.

An experiment was carried out under controlled conditions in the greenhouse to determine whether repeated applications of copper salt sprays would be injurious to the productivity of the soil. It was calculated that 25 lb. of copper nitrate or copper sulphate was the amount of chemical required to

*Treatments made in 1932 with weak solutions of sodium chlorate were not satisfactory. The crop was seriously injured, resulting in a considerable loss of yield of grain at harvest.

Another chemical, ammonium thiocyanate, under trial for the first time this season, ruined the crop completely.

In all other respects, the 1932 experiments confirm the writer's observations recorded in this paper.

spray one acre of weed-infested land. In consequence, provided there was no leaching of the chemical, no oxidation and no assimilation by the plants, there would be after ten treatments 250 lb. of pure copper salt per acre, after 40 treatments 1000 lb., etc.

The results, contrary to expectation, indicated that even the presence of 2500 lb. of copper sulphate, or copper nitrate per acre, was not injurious to plant growth. On the contrary, as indicated in Tables II and III, the chemical treatment stimulated plant growth, resulting in greater length of straw and longer heads. Somewhat similar results were obtained by Dr. J. D. Newton, at the University of Alberta.

TABLE II
EFFECT OF COPPER SULPHATE ON THE HEIGHT AND LENGTH OF HEADS OF WHEAT PLANTS

Copper sulphate per acre, lb.	0	25-175	250-500	750-1000	1250-1500	1750-2000	2250-2500
Av. height of cereal, in.	20.7	21.7	21.9	22.2	22.0	22.6	23.7
Av. length of heads, cm.	4.50	5.20	5.29	5.50	5.82	6.12	6.17

Copper nitrate caused still greater stimulation owing probably to the action of the nitrate.

Iron sulphate and sodium chloride are less effective for weed control in western Canada than in districts with more humid climate and higher concentrations are required. This, added to a much higher cost of the chemicals because of transportation charges, makes their use in Saskatchewan prohibitive.

It can be seen that it is very difficult and indeed nearly impossible to state offhand the amount of spray and the degree of chemical concentration which will give best results in all cases. This depends much on the water supply, the degree of weed infestation, the state of development of both the weed and the crop at treatment, etc. The total amount of pure chemical applied per acre, however, is more stable and if, for instance, in the case of sulphuric acid an 8% solution by weight is suggested in amounts of 75 gal. per acre, it is possible to vary either way according to local conditions, using 100 gal. of a 6% solution or 50 gal. of a 12% solution. These concentrations are by weight.

The Sprayer

The results depend to quite an extent on the spraying equipment. Cheapness and efficient application of the spray are factors to be considered when purchasing a sprayer for field use. Efficient application of the spray requires a high pressure (at least 100 lb.), an even pressure and an even application. Cost of application depends on capacity and construction of sprayer as well as economy of labor. High and even pressure are essential. Bolley (10) in 1908 wrote, "Ordinarily, the pump should deliver a pressure of 100 pounds or more per square inch, and there should be a gauge to show the pump pressure. I have not seen a machine which did good work with a lower pressure." This can easily be understood since, unless the spray is very fine, much of the solution will drop to the ground. If the water used in making the solution is not

strained properly, some of the nozzles become obstructed resulting in strips of untreated weeds. The height of the boom or spraying bars above soil level must be regulated according to the height of the weeds; if it is too low, the tips of the taller weeds will escape treatment.

Sulphuric acid sprays can be used only in specially constructed sprayers where all parts coming in contact with the liquid are brass, lead, wood or rubber.

Weather Conditions

The lethal effect of a chemical spray depends to a great extent on the weather conditions. Aslander (5), spraying mustards with sulphuric acid solutions, showed that:

1. The weeds were killed under all moisture conditions but the results were best in dry air.
2. Increase in temperature gave increased efficiency.
3. Artificial rain applied one hour after treatment even with a weak solution did not decrease the action of the spray.

Dealing with iron sulphate, Aslander, in the same article, showed that contrary to sulphuric acid it was most destructive in an atmosphere of 100% relative humidity. Under such conditions a 5% solution applied on mustards in the greenhouse completely killed the plants in 24 hr. On the contrary, with a relative humidity of from 30 to 60% the solution sprayed upon the plants rapidly evaporated and crystals were formed on the surface of the leaf without injury to the plants. Solutions up to 15% gave similar results. Low atmospheric humidity is usual under western prairie conditions during the daytime, and Aslander's results well illustrate why it is found necessary in Saskatchewan to use a 25% solution of iron sulphate in place of a 10 or 15% solution as recommended by many European investigators.

Evaporation also plays some part as shown by the recent studies of Bissey and his coworkers (6). At a given temperature iron sulphate is more effective when dried slowly than quickly, while the contrary is the case with copper sulphate.

Weather conditions influence the results from chemical treatments in many other ways: they affect the degree of weed infestation of a field before treatment, the relative development of both weeds and crop, and their appearance. A heavy rainfall after treatment may cause the germination of a new lot of weed seeds or hasten the recovery of plants seriously injured but not killed.

In southern Saskatchewan in 1930, the amount of moisture stored in the soil was very small and this condition was aggravated by the excessive evaporation resulting from the high winds which prevailed in May and June. Crops and weeds which in general had started normally made little headway except in the depressions where moisture was more abundant. In the higher places, most of the weeds were not able to compete with the cereal; they dried out and disappeared before harvest. In 1931, near Watson, the opposite condition prevailed, moisture was scarce in early spring and the crops remained fairly free of annual weeds until the middle of June, when heavy showers fell in the district.

Soil moisture and temperature play a large part in determining the degree of weed infestation of a crop. In general if soil moisture is plentiful, but soil temperature unsatisfactory for the germination of the cereal (a condition which often prevails when seeding is done too early), the weeds get ahead of the cereal and the reduction in yield at harvest is considerable. If on the other hand both moisture and temperature are favorable for germination of the cereal, most of the weeds will be smothered.

In years of low precipitation and limited soil moisture one of two conditions will be found: the top layer of the ground is dry and at a depth of 2 or 3 in. there is just enough moisture to permit the germination of the cereal and the crop at harvest will be clean. If the soil down to, for instance, 4 in. is too dry for germination of either weeds or crop, the first rain unless unusually heavy will germinate only the weeds near the surface and this crop at harvest will be very weedy and the loss excessive.

The Weed

The method of weed control by chemicals in the crop is based on the relative resistance of plants to chemical sprays. It is thus essential to know what is the susceptibility of both weeds and crops at different stages of development under conditions of soil and climate prevailing in a district.

Aslander (5) showed that under ordinary conditions a sulphuric acid solution penetrates the tissue rapidly, kills the cytoplasm without causing plasmolysis, decomposes the chlorophyll as it unites with the magnesium atom in the chlorophyll molecule, and destroys the chloroplasts. The cell walls are generally not affected except on very young leaves. More acid is needed to kill leaves with thick cell walls (such as in the case of winter annuals) than the thin leaves of young plants. Weeds grown in the greenhouse are more readily killed than weeds grown in the field. Weeds with waxy leaves such as hare's-ear mustard; are more resistant than plants such as common wild mustard and stinkweed. Plants of the grass family whether cereals or weeds are all fairly resistant to weak chemical solutions because their leaves are in most species narrow and waxy and because also, at time of spraying, the growing point is still well protected.

In the case of chemicals which are toxic, action is much less rapid than in the case of sulphuric acid. The essential factor in the susceptibility of the weeds to toxic sprays is ability of the weed to retain the spray for a certain length of time; if the leaves are small or very smooth, the droplets of spray will run off rapidly and the treatment will not be satisfactory. This explains why stinkweed, which is just as susceptible to sulphuric acid sprays as wild mustard, cannot be controlled successfully in a crop with copper sulphate or iron sulphate, while common wild mustard can.

The number of weed species which can be controlled in a crop depends directly on the resistance of the crop, which is greater in Europe where winter cereals are grown. The latter are more resistant to chemical sprays than spring cereals. Under Saskatchewan conditions, it appears that only a few species can be controlled successfully. In wheat, sulphuric acid will control

common wild mustard, Indian mustard, wild radish, stinkweed, false flax, tumbling mustard, and wild buckwheat. New shoots of Canada thistle are somewhat retarded in their development; hare's-ear mustard, ball mustard, cow cockle, lamb's-quarters, Russian pigweed and the grasses, wild oats, darnel, etc., were not controlled under the conditions of the author's experiments. It is thus important that chemical treatments be limited to such fields where weed infestation consists for the largest part of susceptible weeds, for the simple reason that in controlling the susceptible weeds, we stimulate not only the crop, but also the resistant weeds. At Watson, Saskatchewan, in 1931 when wild mustard or stinkweed was associated with either wild oats, lamb's-quarters, or Russian pigweed, there was always an increase in the development of the latter weeds after treatment.

For plot experiments it is best to select fields with only one weed species dominant, for instance, either common wild mustard or stinkweed. Uniformity in the kind of weed infestation however is not enough, and uniformity in the degree of weed infestation of the plots is necessary when it is desired to submit the yield data to biometrical tests of significance.

Let us first consider the effect of variable weed infestation on the yield of harvested grain and then see how variable yields affect the biometrical analysis.

An experiment conducted by the writer both in 1930 and 1931 under field conditions, to determine the effect of variable degrees of wild mustard infestation on the yield of wheat at harvest showed conclusively:

1. That even a light weed infestation causes a reduction in the yield of harvested grain.

2. That an increase in the number of mustard plants per unit area causes a steady decline in the yield of the grain, in spite of the fact that the mustard plants are smaller and set less seed. The results of this experiment for 1931 are summarized in Table III.

TABLE III

EFFECT OF VARIABLE DEGREE OF WILD MUSTARD INFESTATION ON THE YIELD OF WHEAT AND WILD MUSTARD AT HARVEST. WATSON, SASKATCHEWAN, 1931

Group No.*	Average no. of mustard plants per sq. ft. area	Wheat, gm. per sample	Mustard		
			Pure seed, gm. per sample	Av. weight per plant	Av. weight seed per plant
1	0-1	1110	43.6	3.18	.65
2	2-3	974	186.4	2.39	.58
3	4-5	942	285.3	2.01	.49
4	7-9	886	342.	1.42	.33
5	12-15	780	461.1	1.16	.26

*Eight plots per group.

The following example will indicate how a variable degree of weed infestation makes it difficult to study the yield data biometrically.

Let us assume two series of plots, treated and untreated, in which all factors are uniform except the number of weeds per unit acre, and that the counts per

square foot at time of treatment were as follows:

Check plots	5	15	21	9	12	1
Treated plots	7	5	14	11	19	4

Let us then assume that in this case the chemical treatment was 100% effective so that after treatment no weeds remained alive in the treated plots. The respective counts after treatment would then be as follows:

Check plots	5	15	21	9	12	1
Treated plots	0	0	0	0	0	0

It is quite conceivable and indeed probable that the difference between average yields taken from the two series of plots would not be statistically significant. The first and last of the treated plots might easily show a decrease in yield, while the individual differences between the other pairs would be so variable that a biometrical analysis would fail to establish confidence in the results owing to the existence of an uncontrollable factor, in this case, the degree of weed infestation.

This can be illustrated by some results obtained at Drinkwater, Saskatchewan, in 1930. A treatment with sulphuric acid of common wild mustard in wheat was made in duplicated plots, one acre in area, and three samples, each of 50 sq. ft., were taken in each plot. By the pairing method, the calculation was as shown in Table IV.

TABLE IV

Samples from		Difference	(Difference) ²	Samples from		Difference	(Difference) ²
Treated plots, gm.	Untreated plots, gm.			Treated plots, gm.	Untreated plots, gm.		
767	798	- 31	961	735	574	+161	25921
1040	1015	+ 25	625	866	936	- 70	4900
1028	736	+292	85264	1197	885	+312	97344

$$\text{S.D. difference} = \sqrt{\frac{215015}{6} - \left(\frac{689}{6}\right)^2} = 150.48$$

$$\text{S.D. mean diff.} = \frac{150.48}{\sqrt{5}} = 67.39$$

$$t = \frac{114.63}{67.39} = 1.8$$

For $n=5$, the t value of 1.8 does not indicate that the difference in yield of grain between treated and untreated plots is significant, although four out of six samples show an increase in yield and the average increase for all samples is 3.68 bushels per acre, or 12.6%. This indicates that unless the weed infestation is very uniform, it is very difficult to show biometrically significant results, and the only remedy is to take a greater number of samples at harvest.

It is difficult to say offhand when the chemical treatment of weed-infested fields should be recommended because this depends on many things; mainly the kind of weed, the degree of infestation, the growth of the weed in relation

to the crop, the kind of crop, the value of the crop, etc. In the case of wild mustard or stinkweed in wheat, a treatment with sulphuric acid will always be a remunerative operation if the infestation is severe.

With moderate infestation, it will be advantageous to make the treatment only if the weed has got an early start and if there is a danger of it overcrowding the cereal. In the case of light weed infestation, treatment should not be made unless the farmer can afford the extra expense of weed control, because it is not likely that the treatment will result in an increased yield.

In years when the price of grain is low, it will be economical to treat only the more heavily infested fields. On the contrary when grain commands a high price on the market, treatments will be advantageous also on less heavily infested fields because a small increase in yield of grain is all that will be required to balance the cost of the treatment.

The Crop

Chemical treatments of weeds in the crop are generally confined to the cereals. In the author's experiments, the cereals ranked as follows for resistance to sulphuric acid sprays:

Resistant	Intermediate	Susceptible
Wheat Oats	Barley Spring rye	Corn

Winter wheat is however more resistant than spring wheat and all cereals are most resistant to chemical sprays at their early seedling stage (three-leaf stage). The fact that barley is less resistant than either wheat or oats was clearly indicated in both the 1930 and 1931 experiments. In 1931 for instance a treatment was made under the most favorable conditions, but there was a significant decrease in yield of the treated portion of the field over the untreated portion. In any case, it is probable that it would not be profitable to spray a field of barley because that crop is able to compete more satisfactorily with the weeds than any of our other cereals.

Other crops than those already mentioned can be sprayed. All grasses for instance are fairly resistant and this permits the control of some weeds in pastures, meadows, lawns, etc. Spraying has been resorted to by gardeners to replace weeding of such truck crops as onions, leeks, etc. Long (47) states that 40 gal. of a 4% solution of copper sulphate per acre does not permanently injure peas, beans, clover, etc. Urban (86) found that red clover (*Trifolium pratense*) is quite resistant to a 5% solution of sulphuric acid (by volume), at the rate of 100 gal. of spray per acre, when the plants have formed their fourth leaf. The spray will, of course, burn these leaves and the upper part of the stem, but the legume quickly recovers. As far as alfalfa is concerned, treatment is also safe after the crop has reached the fourth-leaf stage, but this is somewhat later than in the case of red clover.

At Drinkwater, Saskatchewan, in 1930, successful control of wild mustard

with a 4½% solution of sulphuric acid was obtained in crops of western rye grass, alfalfa and sweet clover sown without a nurse crop, but considerably more experiments are required before treatment of sweet clover, alfalfa or flax can be safely recommended under Saskatchewan conditions.

The cereal should be sprayed before it is in the shotblade and whenever possible in its third- to fourth-leaf stage, when the seedlings recover most rapidly and the weeds are well exposed to the direct action of the spray. In general it can be said that the injury caused by chemical sprays to the cereal is in direct relation to its development, and this is indicated in Table V, which gives the summarized results of treatments of wild mustard in wheat at different stages of plant development, and in Tables VII to XII.

TABLE V
AVERAGE YIELD OF WHEAT FROM TREATED AND UNTREATED PLOTS

Period	Check	Treated with H ₂ SO ₄	Diff. in % of check	Check	Treated with CuSO ₄	Diff. in % of check
June 5-20	224.2	208.7	-7	161.1	218.5	34.5
June 21-July 2	196.1	166.2	-15.2	162.0	172.5	+ 6.5
July 3-21	170.9	120.0	-29.8	172.5	159.5	- 7.5

These treatments are not quite comparable, because the acid solution was actually stronger than the copper sulphate solutions. The data, however, clearly indicate that the amount of injury increases directly with the development of the crop.

In general, treatments made early in fields of wheat or oats heavily infested with wild mustard, stinkweed or some other weed susceptible to chemical sprays, result in increase in the yield of grain at harvest. On the other hand, late treatments cause a decrease in yield of grain for the double reason that the weed has then already caused much damage and the cereal does not recover as well from the injury sustained.

Evidence of the beneficial action of chemical treatments on yield of grain alone can be found in a great many articles on the subject. Reference is made, however, mainly to the work of Korsmo (45) whose summarized results are given in Table VI.

TABLE VI
AVERAGE INCREASE IN YIELD OF WHEAT OBTAINED BY KORSMO IN THE COURSE OF EXPERIMENTAL WORK WITH HERBICIDES IN GROWING CROPS

Treatment with	Number of tests	Average increase in yield in % of untreated
Sulphuric acid, sprayed	676	26.5
Iron sulphate, sprayed	680	23.6
Nitric acid, sprayed	122	23.3
Calcium cyanamide, dusted	630	23.9

Following are tabulated some of the results obtained from spraying experiments at Watson, Saskatchewan, in 1931.

TABLE VII

EARLY TREATMENT OF STINKWEED IN WHEAT WITH SULPHURIC ACID;
8% BY WEIGHT, 75 GAL. PER ACRE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Wheat	701	536	+30.8
Stinkweed seed	15	271.6	-94.5

TABLE VIII

MEDIUM LATE TREATMENT OF STINKWEED IN WHEAT WITH SULPHURIC ACID; 6% BY WEIGHT,
100 GAL. PER ACRE, STINKWEED INFESTATION UNUSUALLY SEVERE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Wheat	396	168.5	+135
Stinkweed seed	34.3	305.8	- 88.8

TABLE IX

LATE TREATMENT OF STINKWEED IN WHEAT WITH SULPHURIC ACID;
6% BY WEIGHT, 100 GAL. PER ACRE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Wheat	249.0	343.2	-27.4
Stinkweed seed	19.1	55.6	-65.7

TABLE X

EARLY TREATMENT OF COMMON WILD MUSTARD IN WHEAT WITH COPPER
SULPHATE; 4% BY WEIGHT, 90 GAL. PER ACRE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Wheat	140.2	122.9	+14.1
Wild mustard seed	0.5	5.8	-91.4

TABLE XI
MEDIUM LATE TREATMENT OF COMMON WILD MUSTARD IN OATS WITH
COPPER SULPHATE; 5% BY WEIGHT, 100 GAL. PER ACRE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Oats	360.7	347.7	+ 3.7
Wild mustard seed	3.8	22.6	-83.3

TABLE XII
LATE TREATMENT OF COMMON WILD MUSTARD IN WHEAT WITH COPPER
SULPHATE; 3% BY WEIGHT, 100 GAL. PER ACRE

	Grams per sample (average weight)		Difference in % of check
	Treated	Untreated	
Wheat	185.4	184.2	+ 0.9
Wild mustard seed	30	47.8	-37.2

Another condition seems necessary to ensure the full benefit of a chemical treatment. This involves the rate of seeding of the crop in a weed-infested field. New weed seeds are always likely to germinate after treatment and unless the rate of seeding is sufficiently heavy, they will not be kept under control. This was evident in 1931 when comparing the amount of mustard seed harvested from treated fields where the rates of seeding were different.

In Europe benefits other than weed control and increased yield of grain are often obtained from chemical treatment. In 1923 Rabaté (69) reported that a field of winter wheat treated with a 10% solution of sulphuric acid (by volume), at the rate of 100 gal. of spray per acre, showed a relative freedom of yellow stripe rust over the untreated field. In 1927 Rabaté (73) wrote, "Our observations both from experimental work and from general field treatments, covering every year since 1912 thousands of hectares, indicate a very marked efficacy of sulphuric acid solutions at strengths of 10 to 14%, 65°Baumé, (by volume) against foot rot of wheat."

Urban (87, 88) made a study of this indirect action of chemical treatment which consists in lessening the injury from plant diseases. He observed that light, temperature and proper aeration are factors favoring the resistance of plants to diseases, and he has shown experimentally that the sulphuric acid treatment favored the factors causing plant resistance to diseases. High atmospheric humidity, for instance, is essential for quick rust development and there is no doubt that in years of abundant moisture a heavy weed infestation and a greater leafiness of the cereal creates within the crop ideal conditions for the development of the disease. On the contrary the removal of the weeds and of a proportion of the cereal leaves causes better ventilation, and improves the

light and temperature conditions within the crop. This lowers the humidity and creates a condition less favorable to the development of rust. Urban explains in the same way the greater resistance of cereal treated with sulphuric acid to *Erysiphe graminis*, *Leptosphaeria*, *Herpotrichoides* and *Ophiobolus graminis*.

Experimental Technique

Very little information is available in the literature on experimental methods for weed control in the crop. The papers which have been published seem to indicate that with some exceptions few attempts have been made to conduct these experiments in a true scientific manner, with due consideration for the many factors which influence them. Besides, most investigators have worked independently. In general, it was necessary for the investigators to rely entirely on manufacturers for free use of equipment and chemical supplies, and on the farmer for free labor and data recordings. A special effort should be made to place weed-control experiments on a higher standard. Biometry must be used to test the significance of yield data but this necessarily involves a complete reconsideration of the experimental methods generally adopted. Whether this can be done in all cases remains to be seen, in view of the presence of such a large group of variable factors as we have attempted to describe in the preceding pages. The Subcommittee on Chemical Experiments of the Associate Committee on Weed Control of the National Research Council has, during the past three years, given some consideration to the question of suitable experimental procedure, and the following technique is to be used by the writer for the 1932 weed control program in Saskatchewan.

Field Technique

Choice of Land

The experimental field must be as uniform as possible in soil and weed infestation. The latter is especially desirable and the weeds should be in the main of one species.

Plot Layout

Three points should be considered in deciding upon a suitable plot arrangement: (1) the object of each experiment; (2) the number of tests in each experiment; (3) the amount of uniform available land.

According to the object of the experiment we can recognize three types as follows:

A. Experiments which require much precision:—example, a study of the comparative value under definite climatic conditions of a few of the most promising herbicides. In this case the plots should be randomized and a large number of replicates used. The plots should be small, 10 by 20 ft., and arranged in a Latin square. When more than eight treatments are to be compared, the Latin square being no longer practical as it would involve too many replicates, we may use the Randomized Block arrangement with four to six replicates according to available space and help.

B. When the information sought is of a more general nature and involves a

great many different treatments:—example, an experiment to determine the relative effect of a chemical when applied at various concentrations and amounts per acre. A satisfactory method is that called the alternate check arrangement. This method has been used both in Alberta and in Saskatchewan in 1931 and consists in grouping the different experiments in a series of experimental blocks, where treated plots alternate with untreated plots. The chief advantages of this method are that it facilitates a direct comparison between treated and untreated plots and gives a fair index of the soil heterogeneity. Treatments should be at least in duplicate within each block.

C. For ordinary field trials, when only one chemical solution is used at a definite concentration, the best procedure may be to spray in strips leaving as many areas of untreated as there are of treated crop.

Border Effect

When the plots are separated by pathways it is generally found that many weeds develop after treatment at the edge of the plots. Care should thus be taken at harvest not to include in the samples portions of the crop within a distance of one foot from a road or pathway.

Counts on Weeds

The efficacy of a chemical solution is often based on the percentage of weed seedlings killed at treatment. The author's experience in Saskatchewan indicates that this method is not satisfactory. In some cases, the percentage of apparent kill at treatment was high with but little reduction in the amount of weed seeds in the crop at harvest. In other cases there were few seedlings killed per unit area but a great reduction in the amount of weed seeds in the crop at harvest. The fact is that complete destruction of the weeds depends on a great many factors, mainly:—the number of weeds per unit area, their development, the relative vigor and density of the crop. It is usually the case, when the weed infestation is severe, that the weeds are not killed outright because the spray does not reach all of the lower leaves. The ultimate result often is about the same as if they had been killed, because it takes nearly as long for the treated weeds to recover as for a new lot of seeds to germinate.

The usual method for counts of weeds is to select, in each plot, two or three square foot areas fairly representative of both weed and crop. These are staked out and the weeds therein counted again a few days after treatment. Better results would evidently be obtained if the counts could be made on larger areas, but when a large number of plots is involved this is out of the question. Difficulty arises in determining the time at which the second count should be made as it depends much on the chemical used; with sulphuric acid the effect on the weed is rapid but not lasting and the plants considered as killed often recover; in the case of iron sulphate the contrary may occur, and plants considered as alive two days after treatment often dry out later with further action of the chemical. Other inconveniences arise when new weeds germinate after treatment and are recorded in the second count.

Sampling

A better method to judge the efficacy of a chemical treatment in the crop

consists of sampling the plots at harvest for both weed-seed content and yield of grain. Two or three representative areas, approximately 25 sq. ft. each, are selected in each plot. First the weeds are pulled by hand, special care being taken to avoid shattering, and then the crop is cut with a sickle. Weeds and crop must be bagged, threshed and analyzed separately. In the case of stinkweed, which shatters quite early, hand pulling should be done from two to three weeks before harvest.

This method of estimating the actual decrease in weed infestation of a crop provides the investigator with much valuable information. It has, however, one disadvantage in that it somewhat underestimates the actual effectiveness of the herbicide. At harvest most of the weeds from untreated plots are matured and their seeds ready to shatter, while weeds left in the treated plots are usually very green. If harvest operations are not too long delayed, these seeds will not shatter to any appreciable extent in the field but will be retained by the plants until threshing.

Recording of Data

The following points are recorded on the standard record sheets designed by the Subcommittee on Chemical Experiments:

Before treatment: size of plot, kind of crop, date of seeding, rate of seeding, type of soil.

At treatment: stage of crop development, stage of weed development, average number of susceptible weeds per sq. ft., average number of other weeds per sq. ft., date of treatment, time of treatment, chemical applied, strength of solution, amount of spray per acre, weather conditions, soil moisture.

After treatment: percentage of susceptible weeds killed, weeds not affected, effect on the crop seedlings.

At harvest: height of crop, date crop matures, height of weeds, yield of grain, yield of weed seeds.

After harvest: weight of 1000 kernels of cereal, size of kernels, general appearance of kernels, occasional milling and baking tests.

In 1930, wheat from plots treated with sulphuric acid, copper sulphate, copper nitrate and sodium dichromate was tested by Dr. Larmour at the University of Saskatchewan, for its milling and baking quality. The results clearly indicated that while the treatment often reduced the size of the kernels, it had no effect on the milling and baking quality of the grain.

Interpretation of Yield Data

The biometrical method used to interpret yield data depends on the plot arrangement. The author has suggested four types of plot arrangement and accordingly four different biometrical methods for analyzing the data. The Latin square and the Randomized Blocks arrangement present no difficulty and may be treated by the method described by Gouliden (31, 32).

With the alternate check system the significance of yield data may be established by taking differences between paired values directly, as suggested by Student, or from the probable error in percentage of the mean of all the

checks in an experiment. The first method seems preferable and is illustrated below:

Three samples, including all weeds and crop from 25 square foot areas, are taken in each plot. We have within each block every treatment in duplicate (t and T) and, on the other hand, one untreated plot on either side of the treated plots (c *ca* C *Ca*). The significance of yield data is obtained by taking differences between paired values directly, there being altogether twelve pairs of samples for each duplicated treatment as follows:

t_1	c_1	t_1	ca_1	T_1	C_1	T_1	Ca_1
t_2	c_2	t_2	ca_2	T_2	C_2	T_2	Ca_2
t_3	c_3	t_3	ca_3	T_3	C_3	T_3	Ca_3

c_3	t_3	ca_3			C_3	T_3	Ca_3		
c_2	t_2	ca_2			C_2	T_2	Ca_2		
c_1	t_1	ca_1			C_1	T_1	Ca_1		

c	t	ca	x	cb	C	T	Ca	X	Cb
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A similar procedure is used to test the significance of yield data of the other treated plots. In the case of treated plots x and X , the data would be compared to the respective samples in plots ca , cb , Ca , Cb , and so on.

In the case of treatments on large areas, where strips of treated and untreated crop alternate, the significance of yield data is also obtained by taking the difference between paired values directly. The number of paired samples required depends much on the evenness of the field and in some cases biometrically significant results may be obtained with but a few samples. In general, however, the more samples that are taken, the more reliable are the calculated values of the probable error, and it might be well to plan having at least twenty paired samples for each treated field.

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A NEW STEM-END ROT OF POTATO¹

BY W. R. FOSTER² AND H. S. MACLEOD³

Abstract

A new disease of potato, a hard, dry, corky, stem-end rot with a sharply defined margin, is reported from the coastal area of British Columbia. Stem-end hard rot is the name suggested for this disease. A fungus, *Phomopsis tuberivora* Güssow and Foster, isolated from rot lesions, reproduced typical symptoms of the characteristic stem-end rot. The optimum temperature for the growth of the causal organism is between 20° and 25° C. The optimum hydrogen ion concentration for the growth is approximately 6.5.

Between 38° to 40° F. (3.34 to 4.45° C.) the usual storage temperature for potatoes, the disease makes little progress. Dipping the diseased potatoes in mercuric chloride, 1:1000, was not an effective control measure. Evidence of field experiments indicates that diseased tubers should not be used for planting purposes.

Introduction

In October, 1930, Mr. H. S. MacLeod directed the senior author's attention to what appeared to be a new characteristic stem-end rot of tubers. It was observed in the following varieties: Irish Cobbler, Green Mountain, Early Ohio, Bliss Triumph, Netted Gem, Early Epicure, Columbia Russet and Early St. George, from the coastal area of British Columbia, Vancouver Island and Fraser Valley. This disease has not been reported from any sections of the Interior. Stem-end hard rot is proposed as the name for the disease.

Symptoms of the Disease

The tubers become infected at the stem-end where a hard, dry, corky rot develops (Plate 1, Figs. 1 to 7) resulting in a slightly sunken circular lesion with a sharply defined margin. The general color of the lesion is wood brown (2), with a glistening margin of light neutral grey. On mature tubers the lesions vary in size from mere pin heads to those involving the whole tuber. Often many small immature tubers are found completely mummified early in the season. Small, brown or black raised bodies often break through the epidermis of the lesion. A typical longitudinal section through a lesion shows a dark, corky zone extending towards the centre of the inner medulla, producing a conical hard rot with a definite margin.

Etiology

Isolation

In collaboration with Dr. H. T. Güssow the brown to black bodies breaking through the epidermis were determined to be the fruiting bodies of a hitherto undescribed species of *Phomopsis* to which the name *tuberivora* has been given (1).

Pathogenicity

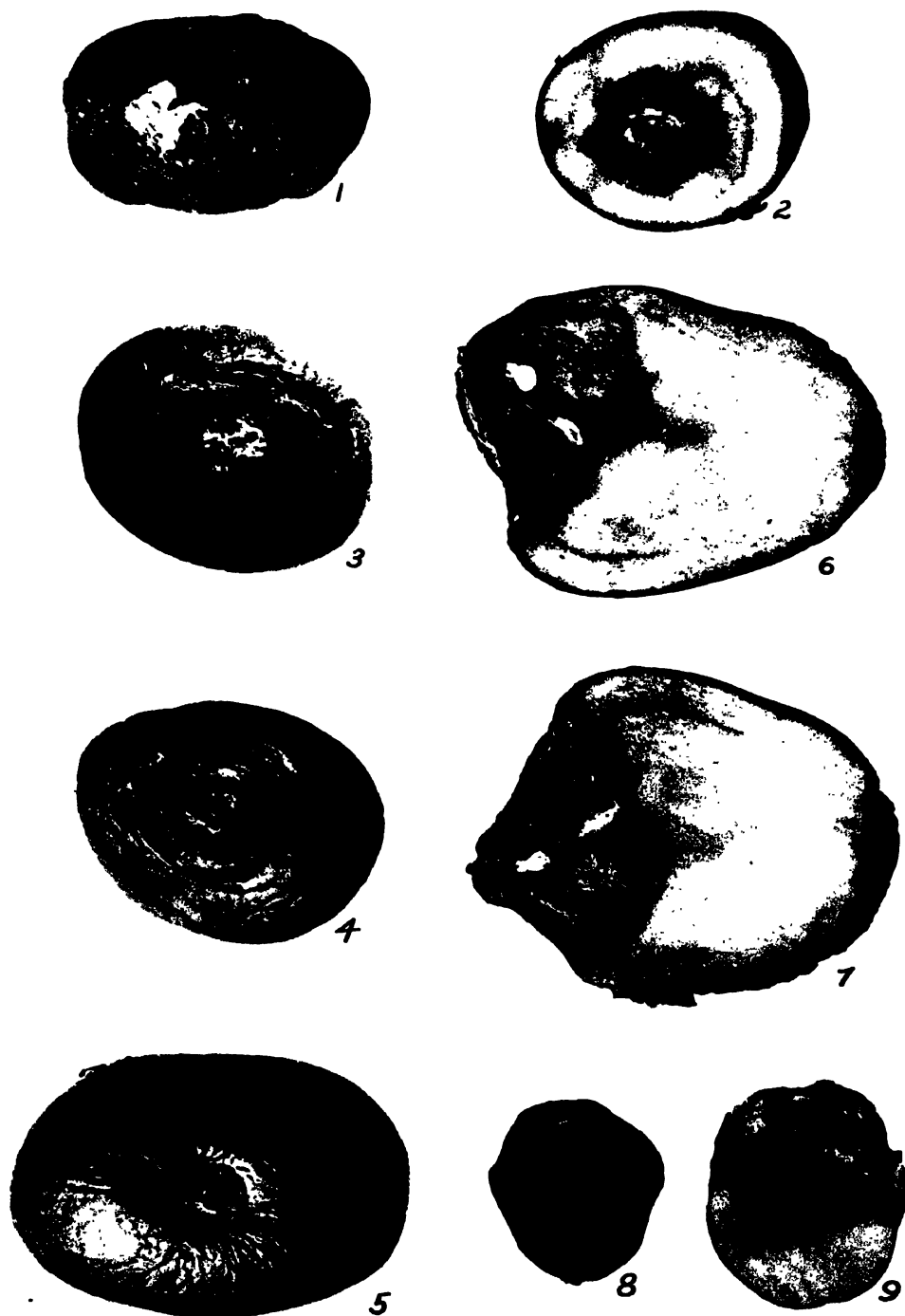
Eight potatoes of each variety representing five tuber-groups were inoculated with mycelium from a single-spore culture of *Phomopsis tuberivora*, and

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Contribution from the Provincial Plant Pathological Laboratory, Saanichton, British Columbia.

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FIGS. 1-7. Stem-end hard rot of potato. FIGS. 8 AND 9. Potatoes inoculated with *Phomopsis tuberivora* Güssow and Foster.

typical symptoms of the disease obtained. Most of the inoculations were accomplished by wounding at the stem-end with an $\frac{1}{8}$ -in. cork borer, followed by the insertion of mycelium from a single-spore culture under aseptic conditions. The wounds were protected from contamination by non-absorbent cotton. Later, it was found that wounding was not necessary. The data with reference to the inoculations are shown in Table I, and the characteristic symptoms obtained from artificial inoculation are illustrated in Plate I, Figs. 8 and 9. These developed in only four of the varieties, *viz.*: Irish Cobbler, Green Mountain, Early Ohio and Bliss Triumph, and not in Netted Gem. However, in the field, this variety proved to be susceptible, and in addition, Early Epicure, Columbia Russet and Early St. George.

TABLE I
RESULTS OF INOCULATING FIVE VARIETIES OF POTATOES WITH *Phomopsis tuberivora*

Treatment	Group	Variety	Result
Check	Irish Cobbler	Irish Cobbler	No lesion
Inoculated	Irish Cobbler	Irish Cobbler	Typical lesion
Check	Burbank	Netted Gem	No lesion
Inoculated	Burbank	Netted Gem	No lesion
Check	Green Mountain	Green Mountain	No lesion
Inoculated	Green Mountain	Green Mountain	Typical lesion
Check	Ohio	Early Ohio	No lesion
Inoculated	Ohio	Early Ohio	Typical lesion
Check	Triumph	Bliss Triumph	No lesion
Inoculated	Triumph	Bliss Triumph	Typical lesion

Environmental Factors

Temperature Studies with Phomopsis tuberivora

A culture of *Phomopsis tuberivora* was grown on potato-dextrose agar and exposed to temperatures ranging from 5° to 40° C. The results are shown in Table II.

TABLE II
REACTION OF *Phomopsis tuberivora* TO TEMPERATURE

Temperature, °C.	5	10	15	20	25	30	35	40
Diameter*, mm.	4	7	33	47	60	12	3	1

*Average diameter of three colonies incubated for three days.

The figures in Table II indicate that the optimum is between 20° and 25° C. These data support the authors' observation that the progress of the disease is very slow at proper storage temperature for potatoes, 38° to 40° F.

Hydrogen Ion Studies

The *Phomopsis tuberivora* was grown in petri dishes on media prepared by the method outlined by Webb and Fellows (3).

The hydrogen ion determinations were made immediately after the plates were poured, at temperatures of, 15° to 16° C., by means of a hydroquinone potentiometer.

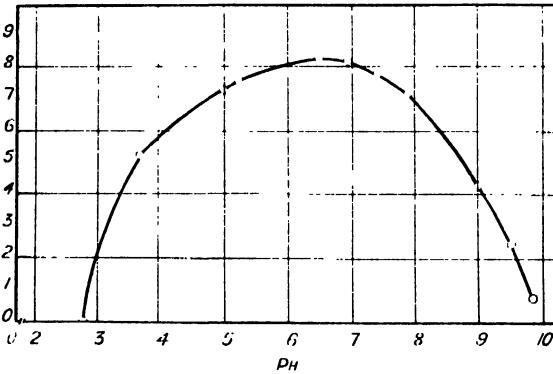


FIG. 10. Growth in centimetres of *Phomopsis tuberivora* at different hydrogen ion concentrations at 25° C.

The results of five days growth are shown in Table III and Fig. 10. Determinations of the pH at the end of the experiment showed that little change of the hydrogen ion concentration had occurred.

The results indicate that the optimum hydrogen ion concentration is approximately 6.5. In 1931 and 1932 the authors found tubers infected with stem-end hard rot in acid soil only.

TABLE III

REACTION OF *Phomopsis tuberivora* TO DIFFERENT HYDROGEN ION CONCENTRATIONS AT 25° C.

pH	2.8	3.7	5.0	5.2	6.5	6.9	7.4	7.9	9.0	9.5	9.8
Diameter,* mm.	0	52	72.5	75.7	82.5	81.8	77.7	71.3	43.2	24.2	6.5

*Average diameter of three colonies for each pH.

Control

Preliminary control investigations were carried on by Mr. H. S. MacLeod.

Diseased and healthy Early Ohio potatoes selected from the same source, were immersed in mercuric chloride (1:1000) for 1½ hr. and planted in the field in uniform soil which had not been planted with potatoes for many years. The

TABLE IV

EFFECT OF TREATING EARLY OHIO POTATOES INFECTED WITH STEM-END HARD ROT WITH MERCURIC CHLORIDE (1:1000)

Treatment	Crops per acre, bushels		Diseased hills, %
	Healthy tubers	Diseased tubers	
Healthy tubers, not treated	312.1	0.2	2.5
Healthy tubers, treated	303.0	6.0	5.0
Diseased tubers, not treated	236.3	21.0	35.0
Diseased tubers, treated	156.0	63.0	80.0

first year's results shown in Table IV indicate that this method of control was not effective, as the percentage of diseased plants was higher from the treated than from the untreated seed. The bushels per acre (Table IV) are based on the yield of only one row of 40 hills, for each treatment. The planting of healthy tubers or sets with affected portions cut off gave a practical control in this experiment.

Summary

1. A new stem-end rot disease of potato has been described.
2. *Phomopsis tuberivora* Güssow and Foster, isolated from rot lesions, reproduced typical symptoms of the characteristic stem-end rot.
3. Stem-end hard rot is the name suggested for this disease.
4. The optimum temperature for the growth of *Phomopsis tuberivora* is between 20° and 25° C.
5. At the storage temperature for potatoes, between 38° to 40° F. (3.34 to 4.45° C.), the disease makes little progress.
6. The optimum hydrogen ion concentration for growth of *Phomopsis tuberivora* is approximately 6.5.
7. The mercuric chloride treatment for potatoes was not an effective control measure.

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THE INFLUENCE OF HELMINTH PARASITISM ON THE ABUNDANCE OF THE SNOWSHOE RABBIT IN WESTERN CANADA¹

By R. V. BOUGHTON²

Abstract

The results are given of a survey of the helminth parasites of 420 rabbits (*Lepus americanus*) from the province of Manitoba. The survey yielded three species of Cestoda, two larval and one adult form, five species of Nematoda, two of which are regarded as new to science, an unidentified Acanthocephalan, and several species of the protozoan genus *Eimeria*. No Trematoda were found.

The biology and pathogenicity of the helminth parasites suggest that the three most dangerous parasites to the health of the rabbit population are *Nematodirus triangularis*, *Synhelocaulus leporis*, and *Eimeria* sp. A definite correlation between the percentage of rabbits infested by parasites and the meteorological conditions in the different soil areas of the province appears to exist.

It has been known for at least a century that *Lepus americanus*, the snowshoe rabbit of western Canada, is subject to periodic fluctuations in abundance, and that such fluctuations are paralleled by similar fluctuations in the abundance of animals which prey principally upon the snowshoe rabbit, such animals, for example, as the Canada lynx (*Lynx ruffus* Gueldenstadt), the fox (*Vulpes fulva* Desmarest) and the coyote (*Canis latrans* Say).

Hewitt (7), from a study of the fur returns of the Hudson Bay Company, asserted that the periods of maximum abundance occurred in the following years: 1845, 1854, 1857, 1865, 1877, 1888, 1897, 1905 and 1914. This gives an average periodic cycle of 8.5 years, which approaches the prevailing popular idea of a seven year cycle for this animal. A maximum abundance however does not occur over the whole country in the same year; such abundance is usually regional in character and the period of general abundance may extend over several years.

Although these fluctuations in abundance of rabbits can be roughly forecasted, it would appear that between the years of abundance there are years of local scarcity. This was the view held by Seton (12) who collected many data in regard to the periods of increase and decrease of rabbits in different areas of western Canada. Seton places the following years as years of rabbit maxima in western Canada:

Lake of the Woods, 1856 (Hind).

Upper Assiniboine, 1857 (Hind).

Portage La Loche, 1875 (J. Macoun).

Shoal Lake and Stony Mountain, 1883-4 (J. H. Cadham).

Red River and Assiniboine Valley 1886-7.

Shoal Lake, 1893-4 (W. G. Tweddell).

Central Manitoba, 1894.

Northern British Columbia, 1872 (J. Macoun).

MacKenzie River Valley, 1903-4.

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Contribution from the Department of Zoology, University of Manitoba, Winnipeg, Manitoba, with financial assistance from the National Research Council of Canada supplemented by the resources of the Department of Zoology, University of Manitoba.

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These fluctuations represent without doubt corresponding fluctuations in rabbit reproduction. The home range of the rabbit is restricted, in many cases to 30 or 40 acres (12), so that migrations of rabbits from area to area cannot be regarded as an important factor in the production of local variations in area population. It is more likely that the fluctuations are due to effects of epidemic disease. Such epidemics are usually associated with overcrowding, although not directly caused by it. The number of rabbits in an area usually increases over a period of years, and finally reaches a maximum; the year of maximum abundance is then followed by a period of epidemic disease, which diminishes enormously the rabbit population of the area. In all cases of observed epidemics among wild rabbits, no single disease has been found totally responsible for the epidemic. Most of the evidence concerning such epidemics is circumstantial, for, in some cases, dead rabbits are found in large numbers, while in other cases, although there is an appreciable decrease in the number of rabbits, few dead ones can be found.

Such evidence as exists with regard to epidemics among rabbits in western Canada is very scanty. Seton (12) records a number of examinations of rabbits that had apparently died during an epidemic. In a number of cases death was found to be due to gastric and intestinal catarrh, with small hemorrhages throughout the digestive canal, caused by roundworms. Another apparently died from a heavy infestation of *Multiceps coenurus*. Others have been reported as having died from enteritis, tuberculosis, pneumonia, and diarrhea. The data available seem, in fact, to indicate that rabbit epidemics are not due to a single infestation or disease, but to the total influence of a number of infestations and diseases, intensified to epidemic proportions by overcrowding.

Although many parasites of rabbits in North America have been recorded, there is very little evidence as to their incidence of infestation. No general helminthological survey of any species of rabbit has been hitherto attempted. D. Dilwyn John (10) made a survey of 516 rabbits in the Aberystwyth area in Wales, but his published data refer only to the incidence of infestation of the Cestodes. A list of the helminth parasites of *Lepus cuniculus* in Great Britain has also been recently published by the Imperial Bureau of Agricultural Parasitology (8).

The results put forward in this report are based upon an helminthological examination of 420 individuals of *Lepus americanus* Erxleben, carried out during the period December 1930 to December 1931, the material being obtained from 32 localities within the boundaries of the province of Manitoba.

Material and Methods

The most important preliminary decision to be made with regard to the actual survey itself was the number of rabbits that should be examined, the intervals at which these examinations should take place, and the area to be covered. The ideal survey, of course, should consist of a monthly examination of a number of rabbits from a large number of localities representing the whole of the province. An alternative method is the monthly examination of as

many animals as possible from just one locality. When the area of the province of Manitoba, which occupies 251,832 square miles, is considered it is obvious that the difficulty and cost of transportation and the number of rabbits that would necessarily have to be examined render the ideal method impracticable. The alternative method is also unsuitable in so large an area, owing to the impossibility of selecting one locality topographically typical of the whole area.

Taking into consideration therefore the size of the province and the cost of collecting material over such a large area, it was considered best to restrict the survey to the southern third of the province, an area of approximately 83,000 square miles, and to make two or three examinations from a limited number of locations during the period of the survey.

During the course of the survey dependence had to be placed upon people in various parts of the province for material; in some cases however between periods of examination some of these people moved, while others neglected to send in more than one shipment. As a result the incidence of infestation of a particular area has in many cases been determined for only one period of the year.

During the summer months rabbits could not be sent in for examination, and it was necessary to spend considerable time in the field during that period. In considering the incidence of infestation between different localities it is necessary to base the results upon samples of approximate equality.

The age of only those rabbits that had been born during the summer of the survey could be determined with any degree of accuracy. All animals older than six months were considered as adults.

The autopsy consisted of a systematic search of all the organs for helminth parasites. The examination of the digestive tract was facilitated by forcing tap water through the intestinal region of the gut and decanting the washed-out debris. In the centrifugal examination of fecal material for eggs, a solution of three parts of cane sugar to two parts of water was used. In such a solution eggs and protozoan cysts can be kept on a slide for some months in excellent condition for examination.

Parasitic infestation could not be determined with certainty during the winter by means of fecal tests, because egg laying is then at a minimum and in some cases does not occur at all. This is particularly the case with *Passalurus nonanulatus*, a roundworm of the large intestine.

In the examination the material was removed as soon as possible after the rabbits were killed. Cestodes were washed in water, stretched upon a glass plate and painted with a fixing solution (10% formalin, acetic sublimate, or Zenker's solution) raised to a temperature of 80°C. Another method of fixing cestodes was to flood them, while *in situ*, with water at a temperature of 70°C. By either method the cestodes were killed in a fully extended condition. The material was preserved in either 5% formalin or 70% ethyl alcohol. The nematodes were fixed in 70% alcohol and 5% glycerol raised to the boiling point.

The best stain for the cestode material was cochineal (one part to five parts of water); differentiation was not necessary. For the nematode material, Erlich's hematoxylin was the most suitable stain. The cestodes were cleared in beechwood creosote, while the nematodes were examined in glycerol.

Cultivation experiments were carried out with *Nematodirus triangularis* and *Synthetocaulus leporis*. Attempts were made with unmodified feces on layers of filter paper. All the conditions appeared favorable for development, but in the case of *Nematodirus triangularis* the results were entirely negative; in the case of *Synthetocaulus leporis* however free larval stages were easily procured; the eggs hatched in the water before the fecal material could be emulsified sufficiently to centrifuge. To obtain a photomicrograph it was necessary to treat the feces with full strength formalin, and even this treatment only retarded egg development, hatching taking place within 12 hr.

In the case of *Nematodirus triangularis* ten other cultures in charcoal and humus were isolated after different periods of time varying up to 15 days, but all failed to yield a single larva. Later, unmodified feces were left covered with water, the water was allowed to evaporate, and the mass of fecal material was left to dry for two days. On the fifth day water was added, and a small percentage of the eggs were found to contain fully developed actively moving embryos. Unfortunately the amount of fecal material in this case was not sufficient to determine whether the eggs would hatch, and time did not permit of further experiments. The fecal material was obtained from wild rabbits kept in captivity. The final experiments were carried on during February, and it is possible that those eggs which are laid during the winter do not hatch; to determine this point further experiments will be necessary.

Sufficient experiments were performed, however, to indicate that a free-living larval stage exists in the case of *Synthetocaulus leporis*, and that a similar stage probably exists in the case of *Nematodirus triangularis*. The fact that the eggs of *Synthetocaulus leporis* develop and hatch within such a short time probably accounts for the high percentage of host infestation, and the greater number in the individual host. It is quite possible that the stage of infestation is reached in a much shorter time than in the case of *Nematodirus triangularis*, so that wet or moist conditions would be required only for a short time, in order to permit egg development, hatching and for host infestation to take place.

The Helminth Fauna

The helminths recorded from rabbits, in most cases, are cosmopolitan in distribution; a few however are local. The helminths recorded in the present paper comprise three species of Cestoda, five species of Nematoda, and an unidentified Acanthocephalan. No Trematoda were found. The protozoan genus *Eimeria* was represented by several species. The helminths found may be listed as follows:—

Cestoda

Fam. Anoplocephalidae. *Cittotaenia pectinata americana*. (Goeze 1782 *partim*, Riehm 1881, Stiles and Hassel 1896)

Fam. Taeniidae.	<i>Taenia pisiformis</i> . sp. larva. (Bloch 1780)
	<i>Multiceps serialis</i> . sp. larva. (Gervais 1847)
Nematoda	
Fam. Rhabdiasidae.	<i>Strongyloides papillosus</i> . (Wedl. 1856)
Fam. Trichuridae.	<i>Trichuris leporis</i> . (Froelich. 1789)
Fam. Trichostrongylidae.	<i>Nematodirus triangularis</i> n. sp. <i>Trichostrongylus</i> . sp.
Fam. Metastrongylidae.	<i>Synhetocaulus leporis</i> n. sp.
Fam. Oxyuridae.	<i>Passalurus nonanulatus</i> (Skinker 1931)

The results of the survey are summed up in Table I. In determining the incidence of infestation, it was thought best, in order to obtain the average infestation, to consider, as nearly as possible, equal numbers of rabbits from the different localities. This was done for two reasons: (a) due to the possible effect of the various factors of the environment; and (b) because the percentage of infestation would necessarily be reduced during the breeding season due to the sudden increase in numbers. In other cases the parasites were not found until the survey had been partly completed and, as a result, only generalized statements can be made. The fact that the large majority of the rabbits were frozen when received made it difficult in some cases to detect some of the parasites.

TABLE I
RELATIVE FREQUENCY OF DIFFERENT PARASITES

Parasite	No. examined	No. infested	% Infested
<i>Cittotaenia pectinata</i>	388 (ad.)	1	0.26
<i>Cittotaenia pectinata</i>	32	32	100
<i>Taenia pisiformis</i>	288	42	14.7
<i>Multiceps serialis</i>	288	45	15.6
<i>Strongyloides papillosus</i>	388	3	0.75
<i>Trichuris leporis</i>	288	29	10.07
<i>Nematodirus triangularis</i> and <i>Trichostrongylus</i>	288	73	25.35
<i>Synhetocaulus leporis</i>	30	25	83.3
<i>Passalurus nonanulatus</i>	288	31	10.7
<i>Acanthocephalid</i>	388	2	0.50
<i>Eimeria</i>	50	40	80

The rabbits were caught during four definite periods, the first from Dec. 15, 1930 to Jan. 20, 1931, the second from Mar. 1 to Apr. 1, 1931, the third from July 15 to Aug. 20, 1931, and the fourth from Oct. 20 to Dec. 30, 1931.

A summary of the infestation, during the three periods, of those parasites for which sufficient data are available is given in Table II. In the case of the

nematodes there appears to be a large decrease in the percentage of infestation in the third period, in contrast to the first period, while in the case of the cestodes there appears to be a slight increase.

TABLE II
COMPARISON OF INFESTATION DURING THREE PERIODS OF THE SURVEY

Parasites	Period	No. examined	No. infested	% infested	No. of males	% Males	% Females
<i>Nematodirus triangularis</i> and <i>Trichostrongylus</i>	Dec. 1930	164	47	28.6	31	65.9	34.1
	Apr. 1931	59	17	28.8	11	64.7	35.3
	Dec. 1931	65	8	12.3	5	62.5	36.5
<i>Trichuris leporis</i>	Dec. 1930	164	20	12.2	13	60	40
	Apr. 1931	59	6	10.1	4	66.6	33.4
	Dec. 1931	65	4	6.1	3	75	25
<i>Passalurus nonnulatus</i>	Dec. 1930	164	15	9.15	9	66.6	33.4
	Apr. 1931	59	11	18.6	8	72.7	27.3
	Dec. 1931	65	5	7.7	2	40	60
<i>Taenia pisiformis</i>	Dec. 1930	164	22	13.4	14	63.6	36.4
	Apr. 1931	59	9	14.2	6	66.6	33.4
	Dec. 1931	65	11	16.9	6	54.5	46.5
<i>Multiceps serialis</i>	Dec. 1930	164	21	12.2	12	57.1	42.9
	Apr. 1931	59	12	20.3	6	50	50
	Dec. 1931	65	12	13.4	9	75	25

NOTE:—Number of males examined—164 equals 56.9%; Number of females examined—124 equals 42.1%.

Cittotaenia pectinata americana

(Goeze 1782 *partim*; Riehm 1881; Stiles and Hassel 1896.) See Fig. 1.

Habitat. Small intestine of *Lepus americanus*, from the province of Manitoba. Common in young individuals. Life history unknown.

Specific diagnosis. Cestodes of large size, maximum length being 220 mm., and 7 mm. in breadth. Segmentation beginning almost immediately back of the head, the proglottids rapidly becoming distinct and always much broader than long. Scolex provided with four shallow acetabula. The *anlagen* of the genital organs appear early. Testes appear about 6 mm. back of the head; genital pores double, in the posterior half of the margin. Testes about 0.64 mm. in diameter, numerous, numbering about 112, and confined to the distal half of the segment, posterior to the uterus, and extend across the entire median field passing the ovary on each side of the longitudinal canals, cirrus pouch about 1 mm. in length, extending some distance median of the longitudinal canals.

Ovary, shell gland and vitellogene gland about 1 mm. from the lateral margin. A common transverse uterus to both ovaries. Cirrus pouch, vagina and uterus pass from the median field into the lateral field dorsally of the longitudinal canals and nerves. Ova about 78 μ in diameter (Plate I, Fig. 1).

Taenia pisiformis. sp. larva

(Bloch 1780)

This metacestode is often called *Cysticercus pisiformis* and occurs commonly in the liver and abdominal cavity of the rabbit. Number of hooks on the rostellum 34 to 48. Length of the large hooks 225 to 294 μ . Length of the small hooks 132 to 177 μ .

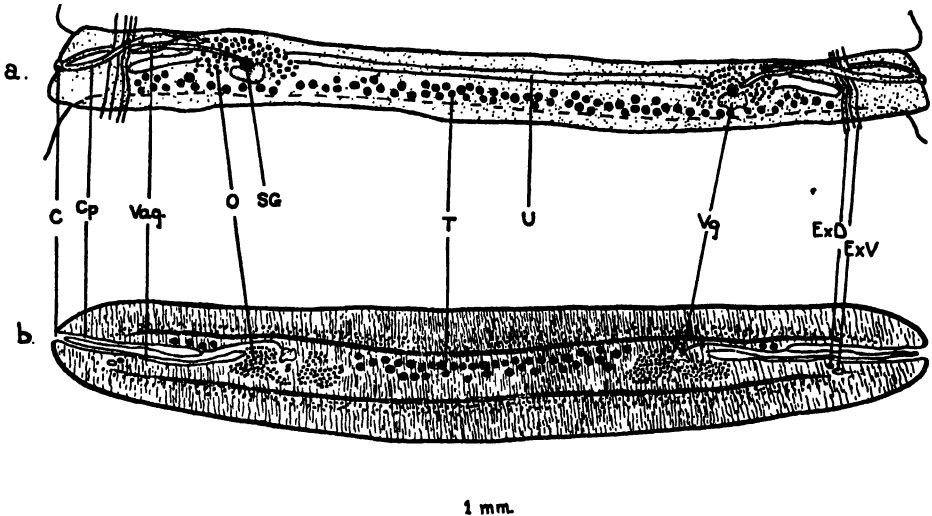


FIG. 1. *Cittotaenia pectinata americana*, mature proglottis; a, dorsal view; b, transverse section; C, genital cloaca; Cp, cirrus pouch; ExD, dorsal excretory vessel; ExV, ventral excretory vessel; O, ovary; SG, shell gland; T, testes; U, uterus; Vag, vagina; Vg, vitelline gland.

Multiceps serialis. sp. larva

(Gervais 1848)

The cystic form of this cestode is often called *Multiceps coenurus*, and occurs commonly in the connective tissue, especially the subcutaneous tissue. Number of hooks 26 to 32. Length of the large hooks 135 to 175 μ . Length of the small hooks 78 to 120 μ .

Strongyloides papillosus

(Wedl. 1856; Ransom 1911) See Fig. 2.

Specific diagnosis. Parasitic generation,—length 3.5 to 6 mm.; thickness 50 to 60 μ . Body filiform, posterior end of the body diminished in size backward beginning some distance in front of the anus, and terminating in a slight tapering tail with rounded tip. Anus 55 to 70 μ from the posterior end. Vulva, a transverse slit with rather salient lips situated 1.6 to 2 mm. from the posterior end of the body. Eggs ellipsoid, with very thin shells 20 by 40 μ to 60 by 25 μ . Not more than a dozen fully formed eggs present in the two uteri at any one time.

Location. Large intestine.

Localities. • Europe, United States, South America, Canada.

Hosts. *Lepus cuniculus*, *Mus decumanus*, *Lepus americanus*.

Trichuris leporis

(Froelich 1789; Hall 1916) See Figs. 3, 4.

Specific diagnosis. Head 17 to 20 μ in diameter, and extending to below the vagina. On each side of the bacillary band an area of delicate cuticular plaques.

Male: 29 to 32 mm. long, with a maximum diameter of about 500 μ . Ratio of the length of the anterior esophageal portion of the body to the length of the posterior portion is 3:2. Spicule 2.88 mm. long, with a diameter of about 8 to 10 μ .

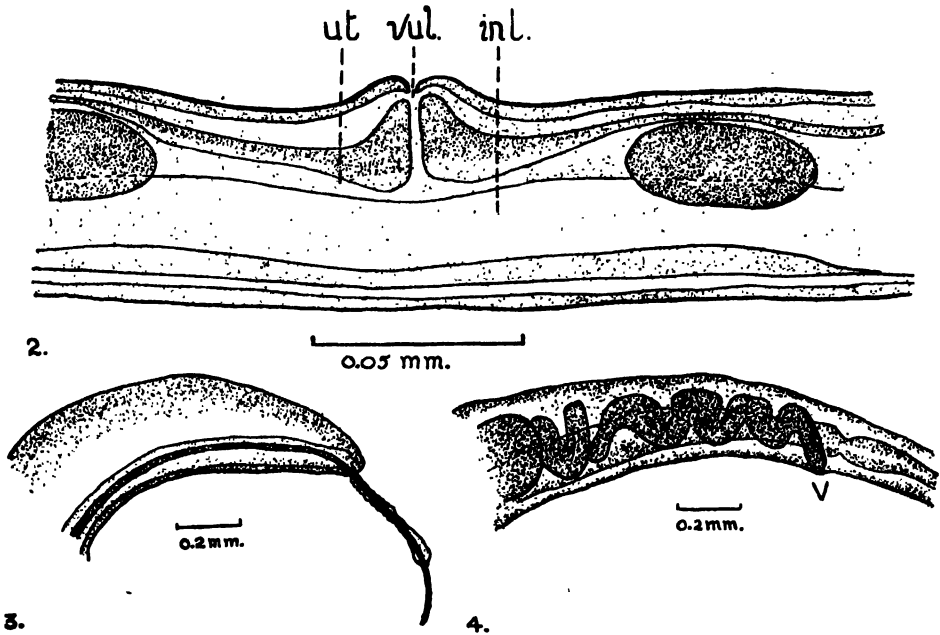


FIG. 2. *Strongyloides papillosus*. Body in region of vulva. int, intestine; ut, uterus; vul, vulva. FIG. 3. *Trichuris leporis*. Posterior extremity of male, showing sheath and spicule. FIG. 4. *Trichuris leporis*. Region of female showing pocketing in vagina. v, vulva.

Female: 24.5 to 32 mm. long, with a maximum diameter of about 910 μ . Ratio of the length of the anterior esophageal portion of the body to the posterior portion 2:1. Vagina appears to comprise a series of pouches or pockets. Eggs 66 to 70 μ long, including the opercular plugs, by 26 to 30 μ in breadth. (Plate I, Fig. 1).

Host habitat. Large intestine.

Hosts. *Oryctolagus cuniculus* (*Lepus cuniculus domesticus* L. c. *ferus*) *Lepus europaeus* (*Lepus timidus*), *Lepus timidus* (*Lepus variabilis*), *Sylvilagus floridanus*, *Sylvilagus floridanus mallurus*. *Citellus citellus* (*Arctomys citellus*, *Spermophilus citellus*), *Lepus americanus*.

Localities. Germany, France, Switzerland, Austria, Italy, United States, Canada.

Nematodirus triangularis. n. sp.

See Fig. 5.

Specific diagnosis. Slender worms of small size. Inflated cuticle of neck asymmetrical, and conspicuously striated.

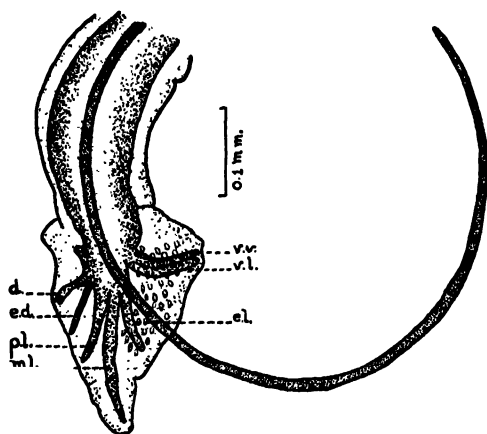


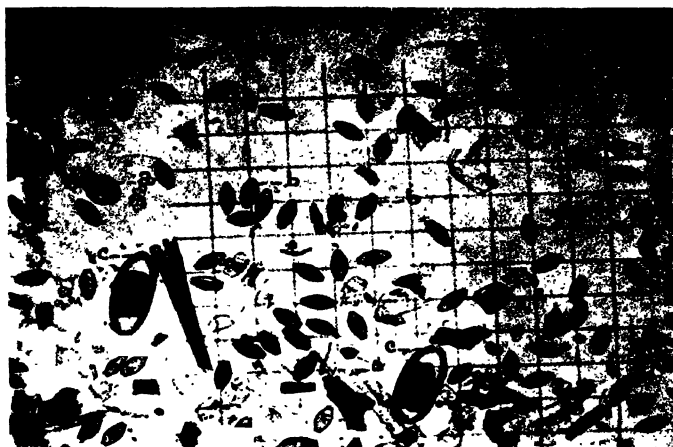
FIG. 5. *Nematodirus triangularis*. Bursa from the right side. d., dorsal ray; e.d., externo-dorsal ray; e.l., externo-lateral ray; m.l., medio-lateral; p.l., postero-lateral; v.l., ventro-lateral ray; v.v., ventro-ventral ray.

Male: 8 to 11 mm. long with a maximum diameter of from 88 to 115 μ . Esophagus 400 to 500 μ in length. Bursa well expanded, its length 260 μ greater than its breadth 185 μ . Ventral lobes set off from the lateral lobes by a distinct notch. Dorsal ray bifurcated to about one-fifth its length. Externodorsal rays long and very slender, about midway between the dorsal ray and the postero-lateral ray. Postero-lateral and the externo-lateral ray much shorter and curving sharply away from the medio-lateral ray. Latero-ventral and ventro-ventral rays in contact for their whole length, curved backward and ending close to the

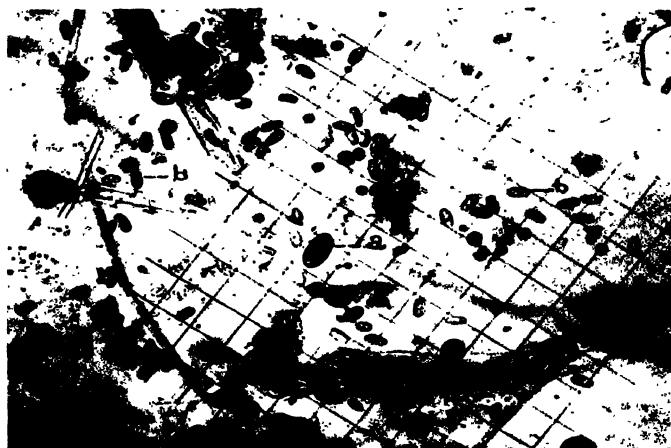
bursal margin. These rays are much more slender than the lateral rays, but much thicker than the dorsal rays. Their length is about one-half that of the medio-lateral rays. Bosses are numerous and small. Spicules deep brown in color, 1.4 to 1.7 mm. long, united for the greater part of their length, and showing distinct striations on the proximal half. Tip of the spicule curved ventrally and ending in a sharp point.

Female: 9 to 13 mm. in length with a maximum diameter of 100 to 160 μ at the vulva. Diameter moderately reduced behind the vulva. Head diameter 35 to 40 μ exclusive of the inflated cuticle, latter well developed reaching a diameter of 50 to 70 μ . Esophagus 450 to 600 μ in length. Tip of the tail truncated and provided with the usual bristle-like process. Anus 70 to 80 μ from the truncated end of the body. Vulva, a transverse slit in the posterior third of the body. Long, oval eggs measuring 175 to 195 μ by 75 to 85 μ (Plate I, Fig. 1) with thin smooth shell of uniform thickness, in various stages of development from six-celled to the morula stage.

This form seems to come nearest in structure to the species *flicollis* (11), which it resembles in general bursal characteristics, in the position of the vulva and of the anus. The male differs however from *flicollis*, in the shape of the bursa, which is definitely triangular, and is sufficient to distinguish the males from any other species of the genus, in the details of the arrangement and relative size of the bursal rays, and in the length and thickness of the body. The writer is therefore of the opinion that this material represents a hitherto unrecorded species, and the species name *triangularis* is suggested.



(1) a. Eggs of *Cittotaenia pectinata americana*. b. Eggs of *Trichuris leporis*. c. Eggs of *Nematodirus triangularis*.



(2) a. Eggs of *Synthetocaulus leporis*. b. Cysts of *Eimeria* sp.



(3) a. Eggs of *Passalurus nonannulatus*.

Type host. Snowshoe rabbit (*Lepus americanus*).

Type locality. Southwestern part of the province of Manitoba.

Location. Duodenum.

Trichostrongylus sp.

(Looss 1905)

This parasite could be identified only as to genus, which is also doubtful, due to the limited amount of material available, and its poor state of preservation.

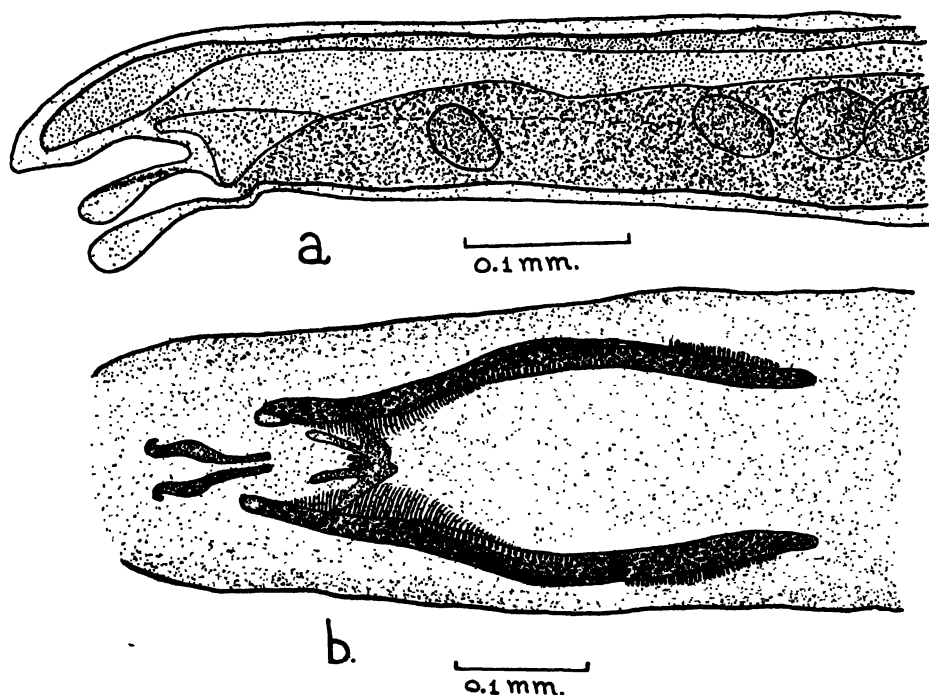


FIG. 6. *Synthetocaulus leporis*. a., posterior extremity of female; b., posterior extremity of the male, showing spicules.

Synthetocaulus leporis. n. sp.

See Fig. 6.

Specific diagnosis. Body filiform. Anterior extremity attenuated and head obtuse. Head with three lips and with six very small circumoral papillae. The slender esophagus is slightly dilated posteriorly. Intestine is dark brown.

Male: 23 to 28 mm. long and 154 to 176 μ thick. The esophagus is 276 to 330 μ long. The small bursa is characteristic of the genus (5). The spicules are 260 to 305 μ long, they are flat, somewhat chitinous rods, broader at the proximal end, and the body of the spicules is marked with a distinct marbling. They are provided with chitinous lateral lamellae, in which are chitinous rods set at right angles to the longitudinal axis of the spicule.* The rods are thicker in the proximal portion of the spicule, in the distal portion the lamellae become wider, and the rods in them become thinner, so that on the distal end they are perceptible only as very fine lines on the thin lateral lamellae. The

lamellae are directed toward one another distally, but curve gradually around the spicule proximally. With the spicules retracted, the unpaired accessory structure lies between them in their distal portion. Its general outline is chevron-shaped with the point of the chevron directed anteriorly, and consists of six to eight sickle-shaped chitinous structures projecting from a centre. The paired accessory structures are located in the bursal region, and are approximately parallel structures 68 to 84 μ long. These structures consist of a broad slightly bent stem, broader toward the distal end, where they curve postero-ventrally.

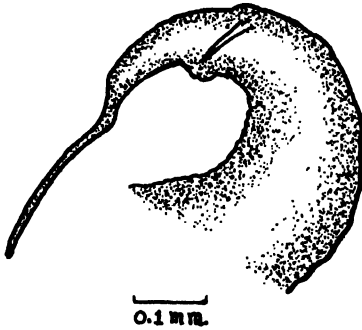


FIG. 7. *Passalurus nonanulatus*. Posterior end of male, lateral view.

Female: 25 to 40 mm. long and 154 to 242 μ thick. The tail is bluntly truncated and the anus is very close to the tip of the tail. The vulva is just anterior to the anus and is 135 to 194 μ from the tip of the tail. The vagina is 1.9 to 2.4 mm. long. Anterior and posterior of the vagina two club-shaped organs occur, which apparently act as ovipositors and are 90 to 110 μ long. There are two convergent uteri. Eggs (Plate I, Fig. 2) are elliptical, very thin shelled, 65 to 75 μ long and 40 to 50 μ thick. They show no trace of segmentation at oviposition.

This species of *Synhetocaulus* seems to come nearest in structure to the species *pulmonalis* (5), which it resembles in general bursal characteristics and size of the vagina. The male differs however in the shape and size of the spicules and accessory structures, the length and thickness of the body and the short esophagus. The female differs in its shorter and more slender body, and the size of the eggs, and in the presence of the two club-shaped organs; the latter characteristic is sufficient to distinguish the female from any other species of the genus. The writer is of the opinion that this material represents a species hitherto unrecorded and the specific name *leporis* is suggested for it.

Type host. Snowshoe rabbit (*Lepus americanus*).

Type locality. Southern portion of the province of Manitoba.

Location. Bronchi, bronchioles, and pulmonary parenchyma.

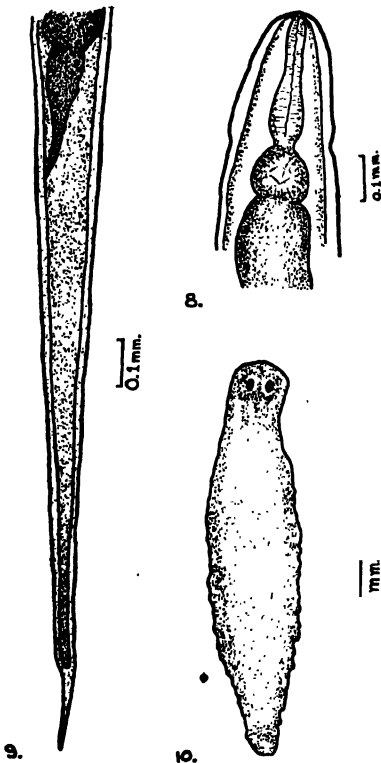


Fig. 8. *Passalurus nonanulatus*. Anterior end of female. FIG. 9. *Passalurus nonanulatus*. Posterior end of female. FIG. 10. *Cittotaenia pectinata americana*. Small immature form.

Passalurus nonanulatus

(Skinker 1931). See Figs. 7, 8, 9.

Male: 3.4 to 4.1 mm. long with a maximum width of about 272 μ . The entire esophagus from 396 to 400 μ long. The bulbar region about 114 to 122 μ long. The spicules are about 115 μ long.

Female: 7.2 to 8 mm. long with a maximum diameter of 400 μ . The esophagus measures 525 to 555 μ in length, the bulb alone being 144 to 160 μ long, and 144 to 176 μ wide. The anus is situated about 1.6 mm. from the posterior end. The slender portion of the tail is 152 μ long. The eggs (Plate I, Fig. 3) are thin-shelled and 90 to 132 μ long and about 60 μ wide.

Hosts. Snowshoe rabbit (*Lepus americanus*), coyote (*Canis lestes*).

Distribution. United States (Cheboygan County, Mich., and Olympia, Wash.); Canada (province of Manitoba).

Location. Large intestine.

Skinker (13) gives the location of this species as the small intestine. The writer, out of a total of 288 rabbits examined, has always found them in the large intestine.

Family Eimeriidae

Reference may be made to the occurrence of oocysts of *Eimeria* in the intestine. There appear to be at least two species present. Oocysts varying from 36 to 52 μ in length by 24 to 27 μ in breadth were found to be the most common. They are ellipsoidal or ovoid in shape, and of a yellowish color. Many of the oocysts show flattening at the micropyle end. A second type found was exceptionally constant in size, measuring 15 μ in length by 11 μ in breadth. The oocysts are almost colorless, elliptical in shape, and there is no flattening at the micropyle end (Plate I, Fig. 2).

Possibly the first form represents *Eimeria stiedae* (Lindeman 1865) and the second form represents *Eimeria perforans* (Leuckart 1879).

The Biology and Pathogenicity of the Leporine Parasites

Although numerous genera of cestode parasites such as, for example, *Cittotaenia*, *Thysanosoma*, *Stilesia*, and *Moniezia*, are parasites in herbivorous animals, in no case has the life history been described. While the present paper does not deal to any extent with the life history of *Cittotaenia*, with the exception of direct feeding experiments, the occurrence and numbers of the cestodes found may help in determining whether or not an intermediate host occurs.

A number of outstanding features have been noticed with regard to the infestation of rabbits with *Cittotaenia*. One is the similarity in the occurrence of *Cittotaenia*, with that of *Moniezia* in the sheep, which has been dealt with to some extent by R. W. Jenkins (1924). Another important feature is the early age at which infestation occurs. Young rabbits from three to five weeks old were found, at autopsy, to be infested with from three to fifteen immature cestodes; all rabbits caught during the third week in July, and which had been among the first litter, probably in May, were found to be infested, the cestodes varying in development from immature forms a few mm. long (Fig. 10) to

mature forms 220 mm. long. Infestation must take place while the young are still on a milk diet or immediately after the change to the herbivorous diet.

The majority of infestations take place from the time the rabbits are three weeks old until they are two months old, but infestation is not confined to this period; immature forms only a few mm. long were found in rabbits that, as far as could be determined, were six months old when caught on October 25. As the first litters are born about the first of May, this period of infestation extends roughly from June 1 to November 1. The period of maximum infestation appears to occur from June 1 to August 1. This period was determined when the maximum number of immature forms were found, and not necessarily when the maximum number of cestodes were found.

Another outstanding feature with regard to the occurrence of *Cittotaenia* is the sudden disappearance of the cestodes in young rabbits during the month of November. From June 1 to about November the incidence in young rabbits is 100%; within a period of three weeks, from November 1 to about November 21 the cestodes are almost totally lost. Of the 420 rabbits examined, 388 were

adults in which infestation was only 0.26%. The rabbits in which the cestodes are lost acquire an immunity which is retained throughout life, because all adult rabbits examined during the time the young rabbits were infested were found to be uninfested. This condition was also supported by examinations during the summer of 1932.

It would appear as if the elimination of the cestodes was due to a change of diet, from soft grass foods to that of bark in the late summer, which takes place about November 1. The adhesive organs of *Cittotaenia*, which consist of four acetabula, are very weak, and it is possible that the mechanical effects due to the movement of the bark cause the cestodes to pass out

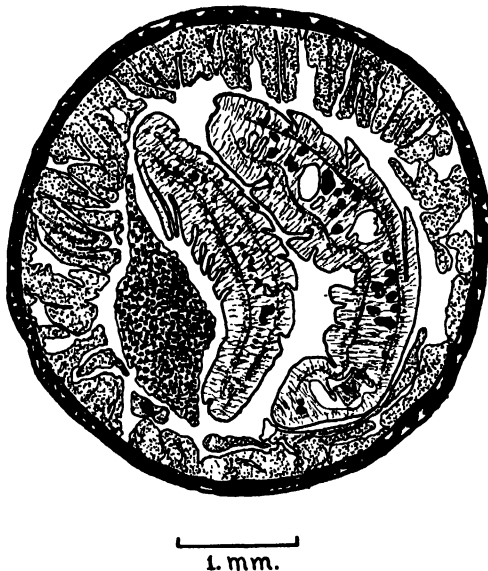


FIG. 11. Figure shows space occupied by an immature form of *Cittotaenia pectinata americana* in the ileum of a rabbit only a few weeks old.

of the host. The ileum has a very small lumen and a number of worms are often found together, or in some cases a single worm is found folded upon itself a number of times (Fig. 11); in either case the gut is often distended in those particular regions. When such a condition exists the worms constitute a serious obstruction to the passage of food material, especially if the infestation is heavy; with the change to a diet of bark the worms pass out of the host.

To determine if this were the real cause of the loss of the worms, a wild rabbit, in which infestation was indicated by the presence of "eggs" in the

fecal material, was kept in captivity from November 1 until February 15, when it died. During this period it was fed upon only a soft diet, and did not pass any worms; at autopsy five mature cestodes were found. Unfortunately these results are based upon a single observation, without a control, which was not secured due to the fact that the worms are lost within such a short time; nevertheless it appears from this experiment and observations in the field that the loss of the worms is due to the mechanical effects caused by the movement of the bark.

Cittotaenia infestation appears to be general all over the province of Manitoba. The topographical characteristics of the area cannot be correlated with the incidence of infestation. As far as can be determined the types of soil, type of vegetation, rainfall or snowfall do not appear to be factors influencing either the percentage of rabbits infested or the number of cestodes found in the individuals. In this respect the cestodes differ from the nematodes.

With regard to the life history two hypotheses exist: (a) some authorities suggest that there is direct infestation without an intermediate host; and (b) other authorities assume that the life cycle is indirect, with the existence of an intermediate host. The latter hypothesis is in accordance with the life history of the majority of cestodes. Direct feeding experiments were tried by feeding rabbits with ripe proglottids that had been kept in water for a number of months, and with "eggs" found in the fecal material. The results were entirely negative.

With regard to an intermediate host three possibilities exist:—

1. The intermediate host may be very common during the period of maximum infestation.
2. The intermediate host may not be common, but may harbor a large number of larval forms.
3. The intermediate host may not be common, but the larval form may be of a multiple type.

It is possible that all three conditions exist. If an intermediate host exists the first condition must exist, because infestation is uniform. If only conditions two and three exist it would be more likely that infestation would be patchy, and that the numbers found in the individual would be large. The second condition may exist but this hardly seems possible, since in this case one would expect to find the worms all of the same age. This is not so; forms are found from a few mm. up to 220 mm. in length. The number found in any one individual has never exceeded 15; in this case it differs from the numbers found in the sheep in which Jenkins (9) has recorded 230 cestodes in the same individual.

The conclusions which may be made in regard to the life history are:—

1. Direct feeding experiments gave negative results.
2. The fact that young rabbits become infested about three weeks after birth, and that infestation is 100%, appears to support the view that an intermediate host exists. With an infestation of only 0.26% in the adults it hardly seems possible that infestation could otherwise spread so rapidly and uniformly.

3. Infestation takes place from June 1 to November 1.
4. The topographical characteristics of the province give no clue to the life history.
5. Examinations of stomach contents of rabbits which harbored a number of very small cestodes in the small intestine gave negative results.

The pathological effects produced by *Cittotaenia pectinata* in adult rabbits can be entirely disregarded since infestation occurs only in 0.26% of those examined. Although infestation in young rabbits is 100% it is of little importance in this case as the cestodes are lost in the fall.

Taenia pisiformis

The larval form of *Taenia pisiformis* of dogs is *Cysticercus pisiformis*; this larval form is usually found in the liver as transparent vesicles, the mesentery, and organs of the body cavity of rabbits. In a few cases the cysts were surrounded by a large mass of calcareous material, lying loose in the body cavity. In two cases partly developed forms were found in the small intestine of the rabbit, these probably represented forms which had failed to bore through the intestinal wall, but instead had proceeded to develop and begin segmentation. Infestation amounted to 14.7%; the maximum number of cysts was 106, the average number being 20.

Multiceps serialis

The larval form of *Multiceps serialis* is usually called *Multiceps coenurus*. These larval forms develop in the rabbit in the connective tissue, under the skin and between the muscles, and are characterized by the production of daughter bladders, which in turn develop numerous scolices. The percentage of infestation amounted to 15.6%, the maximum number of cysts being 14, and varied in size from $\frac{1}{2}$ to $2\frac{1}{2}$ in. in diameter, with a maximum length of 4 in. In four cases cysts were found on the heart, the largest being an inch in diameter. These cysts may have been the larval form of *Multiceps packii* (2) but it was impossible to determine this from the size and shape of the hooks. In one case the lungs were infested by this parasite.

This parasite can no doubt cause sufficient pathological effects to produce death; however the number of cysts or the size of the cysts would have to be considerable. A heavy infestation not only hinders the rabbit in its movements, but also may retard or completely prevent the change in the color of the fur, making it an easy prey to its enemies. Reporting on a specimen that had died, Dr. W. Reid Blair of the New York Zoological Park states*, "Death was due to a generalized infection with tapeworm cysts. These cysts are present in enormous numbers in practically all tissues of the body, the liver, mesentery, diaphragm, spleen, and pectoral muscles being most involved. A multilobular cyst situated over the ribs on the left side was completely encapsulated by the superficial muscles. This cyst contained besides four ounces of clear fluid, about three hundred heads of *Taenia* (mature) every one of which is fully capable, under favorable conditions, of developing into a mature tapeworm".

*See Reference (12).

Coming now to the biology of the nematode parasites found, it is clear that *Strongyloides papillosus* and the *Acanthocephalid* worm occur so seldom as to have no possible influence on host abundance. *Strongyloides papillosus* was found in only 0.75% of the rabbits, and the small size and number found in each infested host (usually four, and in one case ten) indicates that unless there is an enormous increase in years of epidemic, this species is of little importance. The remaining species demand a more detailed discussion.

Nematodirus triangularis and *Trichostrongylus*

These small roundworms are easily recognizable. They are the only roundworms to be found in the small intestine and both male and female are usually found coiled each in a spiral. The worms occur in 25.35% of the rabbits. To make any definite statements with regard to the pathological effects of these worms upon their host is rather difficult, but it is well known that small size in nematodes does not necessarily imply non-pathogenicity. Recent investigations have shown that the members of the superfamily Strongyloidea produce hemolytic and cytolytic toxins (6). Although caution must be used in comparing the effects produced by closely related species of parasites it is possible that the effects produced by *Nematodirus* and *Trichostrongylus* may be similar to those produced by *Ankylostoma* infestation in man, in which the adult worms, by tearing bits of the mucosa from the intestine, produce small hemorrhages, the bleeding being maintained by the excretion of a hemolytic toxin. The worms constantly change their position, and when the numbers are great the loss of blood may be considerable. Providing the numbers are great enough, the total effect produced by the hookworm would be to produce an anemic animal, often retarded in physical and mental development.

Since we are concerned mainly with the lowering of the resistance of the host, the frequency of the worms in the rabbits is of importance. The numbers in any individual never exceeded 60 worms. This number would probably not produce fatal effects. The factors affecting the percentage of infestation and the number of worms found in the individual will be discussed in a later part of the paper.

Trichuris leporis

Trichuris is generally known as the whipworm, and occurs in the large intestine. Ten per cent of the rabbits were found to be infested, but never in large numbers, 29 being the maximum number, so that they could hardly be of any pathological importance, unless the incidence is greatly increased during epidemic years.

Synthetocaulus leporis

This species occurs in the bronchioles and pulmonary parenchyma. The percentage of infestation has not been determined for this worm, because infestation was rather difficult to detect in tissue that had been frozen; however, the percentage is undoubtedly very high since 25 out of 30 freshly killed rabbits examined were infested. The number of individuals present in the lungs has not been determined but 50 or 60 worms were not uncommon in one lobe of the

lung. Infestation is indicated externally by grayish patches on the lungs, indicating the areas in which the worms are localized. The pathological effects produced by infestation with this genus have been summarized by Hall (5) who reviews a study made by Doctor (1907). Hall states: "He finds the initial stages characterized by bronchitis and peribronchitis. Secondly there occurs bronchiectasis, collapse of the aveolar groups, and atelectasis. The changes follow a pneumonic process which is different from others in that it is characterized by extended epithelial desquamation, and a diffuse, progressive course from which it may be known as desquamative pneumonia. This may heal by a regeneration of the denuded epithelium accompanied by hyperemia. If the worm invasion was massive, or if weak respiration or absence of expectoration hinders the elimination of the exudate due to the parasite, there follows a tissue necrosis by progressive caseation, which may show a caseous bronchitis and caseous pneumonia. This may determine extensive destruction of lung tissue or induration." Since this parasite, even at present, which is a time of minimum infestation, must have some effect upon the health of the rabbit population, it appears that in epidemic years, the numbers increase to such an extent that pneumonia sets in, accompanied by other secondary diseases.

Passalurus nonanulatus

Passalurus nonanulatus is a small white nematode sometimes found in large numbers in the large intestine of the rabbit. It occurs in 10.7% of the rabbits examined. In a few cases the large intestine had been found to be almost solid with these worms, rendering it impossible to make an accurate count which sometimes ran into many hundreds. It no doubt feeds upon the food materials in the gut and it does not have any serious pathological effects upon its host, except by the removal or absorption of food or possibly by the secretion of toxins (about which nothing is known).

Eimeria

This coccidium is undoubtedly a dangerous protozoan. It is an intracellular parasite, developing chiefly in the epithelial cells of the small intestine, and may when present in large numbers give rise to acute enteritis. The oocysts are readily detected in the feces. Infestation though quite common was not usually severe; it was certainly present in 40 out of 50 rabbits, and it is quite probable that the percentage of infestation may have been higher. Wenyon (17) in regard to this parasite states, "Young rabbits are especially liable to infection, and they frequently die in large numbers from acute hepatitis, which is caused by the active multiplication of the coccidium in the biliary epithelium. When an outbreak occurs in epidemic form there may be a high rate of mortality. If the animals survive the acute stage, as they not infrequently do, the infection becomes of a chronic type, and it is found that the infection still persists but is limited to certain areas of the liver, where the bile ducts have been dilated to form white nodules, which may have a diameter of half an inch or more. Infection is spread by oocysts which escape into the

intestine and are passed in the feces". In regard to the enteric form the symptoms ascribed to coccidiosis are chiefly of an enteric or dysenteric character.

DISCUSSION

With the exception of *Cittotaenia pectinata* and *Passalurus nonannulatus*, it appears that any one of the parasites discussed is capable of producing sufficient pathogenic conditions necessary to produce an epidemic. The most important of these however are *Nematodirus triangularis*, *Synthetocaulus leporis* and the two species of *Eimeria*. These three forms are undoubtedly capable of producing epidemic conditions. During the survey sufficient numbers were not found to cause epidemic conditions, with the possible exception of *Synthetocaulus leporis*.

Experiments have shown that a free larval form exists in the case of *Synthetocaulus leporis*, and that a similar form probably exists in the case of *Nematodirus triangularis*. In the cases of both these species infestation depends therefore upon the amount of moisture present in the soil. It is also well known that in the breeding of tame rabbits damp or moist conditions favor the development and percentage of infestation of *Eimeria*. It appears therefore that these three parasites are capable of producing pathogenic conditions sufficient to cause disease in epidemic proportions, and that they are all favored by wet or moist conditions. The factors, which include rainfall, affecting the percentage of infestation will be discussed in a later part of the paper.

The Correlation between Roundworm Infestation and Metero-topographical Factors

It is well known that the eggs of parasitic roundworms when outside the host require moisture before development can proceed. Lack of moisture, on the other hand, retards or prevents development, and after a limited time the eggs may fail to hatch even when placed in a suitable environment. Such is the case in forms in which infestation is direct, such as *Trichuris* and particularly in the case of strongyloid worms, which have a free-living larval stage.

Neither the life history of *Synthetocaulus leporis* nor *Nematodirus triangularis* have been completely worked out, but it is definitely known that a free-living larva occurs in the case of *Synthetocaulus* and that possibly a similar form occurs in the case of *Nematodirus*; thus we know that we are dealing with a soil pollution infestation.

The conditions necessary for the development of strongyloid larvae in nature usually are suitable food and temperature, the presence of a certain degree of moisture, and as secondary factors affecting the percentage of eggs that hatch, the type of soil and vegetation.

The domestic range of the rabbit is very small (12). Each individual has its own little territory which varies in extent with the topography. In very thick woods it may not exceed 20 or 30 acres, and in bush country perhaps not more than 50 or 60 acres. Very little migration outside this range takes place, and as a result the rabbit is an excellent animal for the study of the factors which affect the percentage of infestation by roundworms.

In determining the relation of meteorological and soil factors to the percentage of infestation it must be remembered that rainfall and temperature over the area are much more uniform than are the types of soil, which may vary considerably even in a small area. Vegetation also varies with the soil and must therefore be considered as varying within these same small limits. The rainfall is probably more important than the types of soil, since it determines to a large extent the surface soil that exists in any one area.

For convenience of discussion the province of Manitoba may be divided into two main areas; a timbered area lying north and east of the line *ab* (Fig. 12); and a southern portion almost totally without timber or scrub lying south of the same line. The area to the south of this line is divided into five zones; each one constitutes a distinct soil province (4). Zone 4 has been enlarged and Zone 5 reduced an equal amount from that given by Ellis (4).

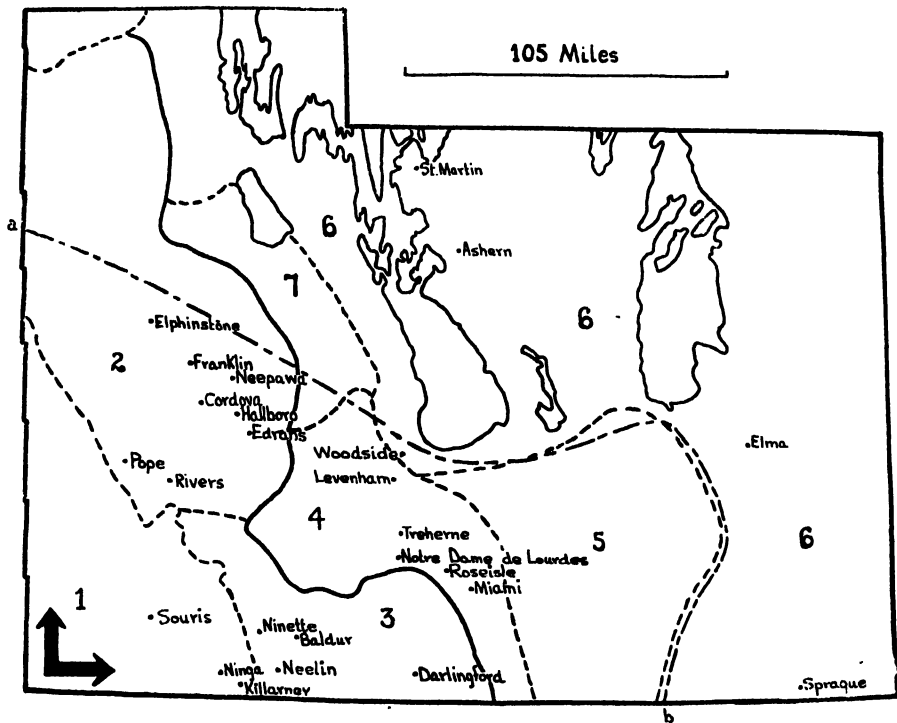


FIG. 12. Map of the lower third of the province of Manitoba, showing the different soil zones. Arrows indicate direction of increase in Nematode infestation and precipitation and a decrease in temperature.

Zone 1 lies in the extreme southwest of the province. The rainfall is below the average for the province; 8.5 in. falls during the period April to July, and 4.6 in. during the period August to October. The temperature on the other hand is above the average for the province. The soil consists of modified drift, sand and dune deposits, with segregated areas of saliferous soil. The vegetation for the most part consists of prairie grasses. The types of soil,

with the low rainfall, hot winds, and moderately high temperature, suggests that this zone would be unfavorable for the development of helminth eggs. This view is supported by the low percentage of infestation in the zone, which is zero in the case of *Nematodirus triangularis*, the hookworm, 5.0% in the case of *Trichuris leporis* and 7.5% in the case of *Passalurus nonanulatus*.

Zone 2 lies to the extreme west of the province and north of Zone 1. The rainfall amounts to 8 in. during the period April to July, and 4.9 in. during the period August to October. The mean temperature is below the average for the province. The drainage is affected by the rolling nature of the country. Basins are formed and, in the years of average rainfall, hold water and form sloughs, which are often surrounded by scrub poplar or willows and offer an ideal habitat for the rabbit. Only the southern portion of the zone has been considered, and this portion consists of two areas; (a) a northern forested area, merging gradually into (b) a southern grass area. Between these two types we find a region in which the trees are invading the prairie, and as a result "islands" of trees occur scattered over the area, giving a park-like appearance. On the whole the soil is close-textured modified glacial drift, and more or less stony. The rainfall, although somewhat below the average for the province, together with the lower temperature, the richer soil, and the greater amount of vegetation provide more favorable conditions for egg development. This is indicated by an infestation of 19.0% in the case of *Nematodirus triangularis*, 8.5% in the case of *Trichuris leporis*, and 12.8% in the case of *Passalurus nonanulatus*.

Zone 3 lies to the extreme south of the province and east of Zone 1. The rainfall is considerably higher than in Zone 2; 9.5 in. falls during the period April to July and 5.2 in. during the period August to October, which amounts to just a little above the average for the province. The temperature is also above the average for the province during the whole of the summer. The soil consists of modified glacial drift and small areas of saliferous soil. The vegetation consists of prairie with fringes of scrub on the banks of the streams. The topography is undulating to rolling and the drainage on the whole is good. The soil is very similar to that of Zone 2 but the increased rainfall, which amounts to 2 in. above that of Zone 2, combined with higher temperatures probably forms more favorable conditions for "egg" development than in Zone 2. Infestation amounts to 27.3% in the case of *Nematodirus*, 9.1% in the case of *Trichuris* and 4.5% in the case of *Passalurus*.

Zone 4 lies approximately in the central part of the area lying south of the line *ab* on the map. The rainfall is higher than the average for the province only during the early part of the summer, but considerably lower during the late summer; 8.9 in. falls during the period April to July, and 4.8 in. during the period August to October. Along the eastern border of the area marshy conditions exist, owing to the subsoil water lying near the surface. The zone is the warmest in the province. The topography varies from level prairie to dune-like hills. The central portion of the area is largely dune sand and grades into fertile sandy planes towards the borders. The vegetation consists of prairie grasses and spruce juniper in the sand hills adjoining the Assiniboine

river. The increased rainfall during the early part of the summer, the water-logged soil and high temperature are favorable for "egg" development, even in a sandy soil (16). The infestation in this zone amounts to 33.3% in the case of *Nematodirus*, 16.6% in the case of *Trichuris*, and 16.6% in the case of *Passalurus*. The comparatively high percentage of infestation in this case is probably due to the fact that rabbits were received from only the borders of the zone, where conditions are more favorable for "egg" development than in the central portion.

Zone 5 lies directly to the east of Zones 3 and 4 and includes the Red River Valley. The rainfall is above the average for the province; 9.8 in. falls during the period April to July, and 5.8 in. during the period August to October. The temperature is higher than the average for the province. The soil is a rich tenacious clay loam of lacustrine and alluvial formation resting on glacial drift. The indigenous vegetation was grassy prairie, with scrub and timber growths fringing the banks of the Red River. The conditions in this zone appear to be more favorable for "egg" development than any of the zones already discussed, but unfortunately no figures are available for the area.

Zone 6 comprises the northern half of the area under discussion, lying north of the line *ab* (Fig. 12). The rainfall is below the average for the province for the early part of the summer, but far above the average for the latter part; 8.1 in. falls during the period April to July, and 6 in. from August to October. The temperature is below the average for the province. The soil is boulder till, modified with lacustrine action, and gravel deposits with a considerable area of peaty soil. The surface soil over a large area consists of "humus", due to most of the area being covered with scrub and timber, which is intermixed with meadow land; a few tamarac swamps occur in the eastern portion and a number of floating bogs in the northwest. The topography is flat and many shallow lakes occur over a large part of the area.

TABLE III
COMPARISON OF INFESTATION DURING THE WINTER AND SPRING OF 1930-31, AND
THE METEOROTOPOGRAPHICAL FACTORS

Zone	Rainfall, in.	Type of soil	No. examined	Percentage of infestation		
				<i>Nemato- dirus</i>	<i>Trichuris</i>	<i>Passalurus</i>
1	13.1	Dune and sand deposits	40	0.0	5.0	7.5
2	12.9	Close-textured drift	70	19.0	8.5	12.8
3	14.7	Glacial drift	22	27.3	9.1	4.5
4	13.7	Sandy loam to dune sand	36	33.3	16.6	16.6
5	15.6	Tenacious clay loam	0	?	?	?
6	14.1	Humus and peat	35	42.9	17.2	6.0

The surface soil, which consists largely of humus, and the shaded moist conditions make the area appear exceedingly favorable for nematode egg and larval development. The zone itself comprises an area much larger than was

covered by the present survey, and only the lower portion skirting the other zones has been investigated. It is probable that in this southern part of the zone the temperature would be higher than that given by Ellis (4). The percentage of infestation in this area amounts to 42.9% in the case of *Nematodirus*, 17.2% in the case of *Trichuris* and 6% in the case of *Passalurus*.

Zone 7 lies directly west of Zone 2 and north of Zone 4. This district has been entirely neglected, not only because rabbits were not procured from the area, but also due to the great complexity of the soil, which includes almost, if not all, the types found in the other zones. The occurrence of the various types of soil produces a corresponding complexity in the types and amount of vegetation. The topography, meteorological conditions and the degree of infestation of the various zones are summarized in Table III.

DISCUSSION

In general the rainfall in the province increases progressively east and north from the southwest corner, the minimum occurring in Zone 2, and the maximum in Zone 5. Temperatures, in general, decrease progressively north and east from Zone 1, the highest mean temperature occurring in Zone 4 and the lowest in Zone 6. The high temperatures that occur in a number of the zones, particularly in Zone 1 and the central part of Zone 4, are detrimental rather than favorable to parasitic egg development, because with good drainage and excessive evaporation unsuitable conditions for egg development are produced. The north and eastern part of the area, although having a lower temperature, have more favorable conditions, due to the humus and wet conditions produced by the vegetation. The soil varies from sandy in the western part of the province to a rich humus in the eastern part.

Experiments carried out by Stoll (16) showed that the greatest number of *Ankylostoma* and *Necator* larvae were produced from a given amount of fecal material when the nematodes were cultured in humus, and lesser amounts were produced in sand, loam and clay, providing moisture and temperature conditions were equal. In the present survey however, it was found that sandy conditions produced the least amount of strongyloid infestation owing to the excellent drainage properties of sandy soil; maximum infestation was found in Zone 6, which is covered to a large extent by humus.

The incidence of infestation in the case of the roundworms depends upon climatic factors, similar to those stated by Spindler (14) for *Trichuris trichura* and *Ascaris lumbricoides*. Conditions in Zone 1 and the central part of Zone 4 are very seldom favorable for nematode egg development, and this was particularly true during the last two years (1930-1931) which have been regarded as dry years in western Canada. There is no doubt that during the past summer (1931) favorable conditions for egg development did not exist in Zone 1 and the central part of Zone 4.

Although conditions appear favorable for egg development in Zone 5, it is possible that, due to the well-irrigated conditions over most of the zone, the incidence of infestation may be lower than in Zones 3 and 4 in which one would expect a lower incidence of infestation.

The following conclusions may be drawn therefore with regard to the correlation between parasitic incidence and climatic conditions:—

1. From the above discussion it appears that there is a definite relation between the incidence of helminth infestation, and the mean rainfall, mean temperature, soil structure and vegetation.
2. The occurrence of a considerable amount of moisture, which appears to be the main climatic factor favoring helminth infestation, may be brought about by one or by both of two factors; either a heavy annual rainfall, or the presence of a large amount of vegetation which would tend to prevent evaporation and conserve the moisture in the soil when the mean rainfall is low.
3. The incidence of infestation appears to be at a minimum in Zone 1, where the surface soil is sandy, and at a maximum in Zone 6, where the surface soil is composed largely of humus.
4. The incidence of infestation in the case of the strongyloid *Nematodirus* agrees with experiments carried out in the laboratory by Stoll (16), except in sandy soil, which is explained by the exceptional drainage property of sand in nature. Infestation in Zone 4 is explained by the ground water coming to the surface towards the borders of the zone, which is the area from which most of the rabbits were received.
5. The number of rabbits examined was small considering the wide area covered by the survey, and to justify definite conclusions it would be necessary to perform autopsies on many thousands of rabbits, but the writer publishes the information available, with the hope that it may lead to further developments.

Acknowledgments

The survey was carried out under the supervision and direction of Prof. R. A. Wardle, to whom acknowledgment is due for his helpful suggestions, kind criticisms, and assistance in the preparation of manuscript.

The author also wishes to express his thanks to Prof. V. W. Jackson and Prof. J. H. Ellis of the Manitoba Agricultural College for their help and suggestions, to Prof. L. A. H. Warren of the University of Manitoba, to the Director of the Dominion Meteorological Bureau at Winnipeg, and to the many people throughout the province who so willingly sent in material.

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REVIEWS AND NOTES

DISCUSSION ON "LONGITUDINAL AND RADIAL VIBRATIONS IN LIQUIDS CONTAINED IN CYLINDRICAL TUBES"

Discussion by C. B. Fisher¹

In Field's analysis leading to the equation of radial motion of the tube wall, the following statement appears (2, p. 132):

"In this relation Young's modulus should be corrected to compensate for the fact that the stresses in the tube wall are rapidly alternating, so that the deformation of the wall does not quite attain the statical value corresponding to the instantaneous distribution of pressure in the liquid. Korteweg suggests the following value be used:

$$(19) \quad E' = E \left(1 - \frac{5h}{6a} \right) \quad "$$

This is hardly justification for any correction of E_1 as the lag in displacement could only be due to the mass of the wall resisting instantaneous displacement. Such an effect occurs, and is taken into account in the differential equation of motion. Korteweg actually justifies Equation (19) in another manner (3, p. 532).

"If we assume that no longitudinal tensions exist, then it is not difficult to take into account, by means of the theory of elasticity, the thickness of the walls, A_1 , in the calculation of E_1 . If E_1 is the ordinary coefficient of elasticity of the tube wall, which also appears in the expansion of a rod of similar material, then

$$E_1 = E_1' \left\{ 1 - \left(1 + \frac{\lambda}{\lambda + \mu} \right) \frac{A_1}{2R_1} \right\},$$

where λ and μ express the two familiar constants (Hilfsgrößen) of the theory of elasticity.

"According to Poisson's hypothesis, $\lambda = \mu$, according to Wertheim, $\lambda = 2\mu$, which is, however, nearer the truth. If we take Wertheim's relation, then

$$E_1 = E_1' \left(1 - \frac{5A_1}{6R_1} \right). \quad "$$

That is, E_1 is corrected, not because of the effects of mass in the tube walls, but because when the tube is expanded, even if by a constant internal pressure, the stress is not the same at every point in the tube wall.

Notice also that Korteweg discarded Poisson's hypothesis, $\lambda = \mu$, and accepted Wertheim's experimental result, which tended to show, $\lambda = 2\mu$. However, later measurements and theoretical studies have disproved the theory that any definite relation exists between λ and μ . This is the attitude adopted by Lamb (4, pp. 114-115) and Rayleigh (6, pp. 251-253). A complete outline of the controversy on this point (the so-called "Multi-constant, rari-constant" controversy) is given by Love (5, pp. 12-14).

¹ *Engineering Department, Northern Electric Company, Montreal. Manuscript received July 13, 1932.*

From results of measurements given by Lamb (4, pp. 114-115) and credited to Everett the following values have been calculated for the factor $\frac{1}{2} \left(1 + \frac{\lambda}{\lambda + \mu}\right)$. Steel, 0.810; wrought iron, 0.775; cast iron, 0.767; copper, 0.878; glass, 0.729. This is the factor taken by Korteweg (and Field) as $\frac{5}{6}$ or 0.833.

There appears to be, however, a further limitation even on these results. The constants given above can be used only if all particles of the tube wall are vibrating strictly in phase with each other. This condition will hold only if the time required for the wave to travel from the inner to the outer wall of the tube, and return, is small in comparison with the period of the impressed harmonic force. Consider for example a glass tube with a wall 0.2 cm. thick. The time for the wave to travel from the inner to the outer wall and return will be about 10^{-6} seconds which may well be of the order of a period of the impressed force.

The tube now becomes a complicated elastic structure with a secondary high-frequency resonance. The analysis leading to the exact value of the natural periods appears to offer considerable difficulty, and seems not to have been given by Lamb, Love, Rayleigh, Crandall or Timoshenko.

The present writer is indebted to Mr. J. P. Aston of Montreal for the translation of Korteweg's paper.

Discussion by Geo. S. Field²

As pointed out by Mr. Fisher, Korteweg introduced a correction to Young's modulus really to take account of the varying stress in the tube wall resulting from its finite thickness, and I regret that in going over an abstract of Korteweg's paper I did not check this point more closely.

I agree with Mr. Fisher that the correction is in any case considerably in error, and I feel now that it might be better to leave Young's modulus uncorrected, awaiting a mathematical examination of all the factors involved. It is certain that the finite velocity of sound in the tube wall will considerably affect the extension of the wall, and in addition the assumption made by Korteweg that no longitudinal tensions exist is not justifiable, since, with a longitudinal wave travelling down the liquid in the tube, the pressure on the wall is varying in phase continuously along the tube.

For thin walls, the factors mentioned above probably have little effect on the actual transmission of sound in the liquid, but when the wall thickness becomes appreciable in comparison with the radius of the liquid column, such effects may become very appreciable as has been shown by experiment (1).

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A MATHEMATICAL THEORY OF THE GROWTH OF POPULATIONS OF THE FLOUR BEETLE, *TRIBOLIUM CONFUSUM*, DUV.

A CORRECTION OF A PREVIOUSLY PUBLISHED FIGURE¹

By JOHN STANLEY²

In a recent paper (1) the writer developed a function, $\theta(T, \gamma)$, descriptive of the number of eggs of age γ at time T and, in a figure, showed the general form of the frequency-distribution surface.

It appears as a result of further thought that the above-mentioned figure (1, Fig. 1) is somewhat in error. This figure was roughly of the form of Fig. 1 of this present note, the line Z being the trace of the plane $\gamma = T$.

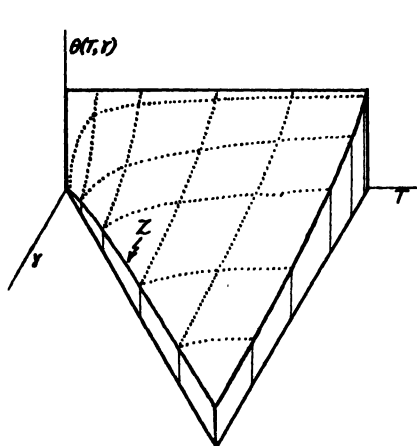


FIG. 1. The frequency-distribution surface $\theta(T, \gamma)$ as previously shown (1).

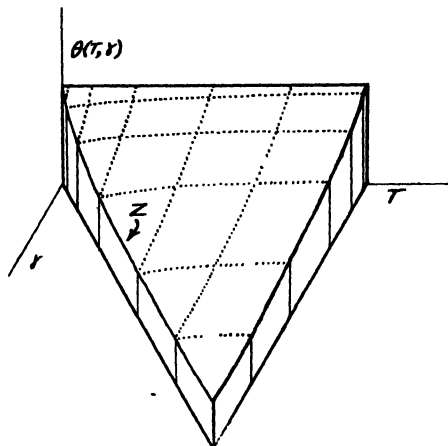


FIG. 2. The frequency-distribution surface $\theta(T, \gamma)$ as corrected.

However, from (1, Equation 28) it is seen that

$$\lim_{\gamma \rightarrow T} \theta(T, \gamma) = \lim_{(T-\gamma) \rightarrow 0} \theta(T, \gamma) = R\epsilon N_{11} \left[1 - \frac{|a|}{b} F(T) \right]^{\frac{H}{|a|}} > 0. \quad (1)$$

This is the equation of the trace Z , and it can readily be shown that

$$\frac{\partial \theta}{\partial T} \left[\lim_{\gamma \rightarrow T} \theta(T, \gamma) \right] = -R\epsilon N_{11} \frac{H}{|a|} \left[1 - \frac{|a|}{b} F(T) \right]^{\frac{H}{|a|} - 1} \left[\frac{|a|}{b} \left\{ \frac{b - |a| F(T)}{c F(T) + d} \right\} \right] < 0, \quad (2)$$

from which it is easily shown that

$$\frac{\partial^2}{\partial T^2} \left[\lim_{\gamma \rightarrow T} \theta(T, \gamma) \right] > 0. \quad (3)$$

Thus, the trace Z should be as shown in Fig. 2, rather than as shown in Fig. 1.

Reference

1. STANLEY, J. A mathematical theory of the growth of populations of the flour beetle, *Tribolium confusum*, Duv. II. The distribution by ages in the early stages of population growth. Can. J. Research, 7: 426-433. 1932.

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RAMAN EFFECTS WITH LIQUID AND SOLID CARBON DIOXIDE¹

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Abstract

This paper describes in detail methods of obtaining clear liquid carbon dioxide and clear solid carbon dioxide in tubes suitable for light-scattering experiments. A description is given of the new type of mercury arc employed as a source of incident light.

For the first time, Raman spectra of carbon dioxide in the liquid and solid states are obtained, and compared with the known Raman spectrum of the substance in the gaseous state.

Carbon dioxide is the first substance for which Raman spectra have been obtained for all three states.

Introduction

Among problems of interest to physicists at the present time is that of the structure of polyatomic molecules. Triatomic molecules, especially, have been the subject of recent detailed analyses by many workers in the field of molecular spectroscopy.

A study of the Raman effect of the substance under consideration provides one of the most powerful means of attacking this problem, for such a study yields valuable information concerning the fundamental vibrational frequencies of the molecules. Considerable work has been done on the Raman effects of a number of triatomic compounds, some of which were in the liquid and some in the gaseous state. Very few investigations have been made, however, with the substances in both the liquid and gaseous phases, and as far as we are aware, none have been studied in the solid, liquid and gaseous states.

In this paper an account of our investigations of the Raman effect with carbon dioxide in the liquid and in the solid state is presented and our results are compared with those obtained for the gas by Dickinson, Dillon and Rasetti (2).

¹ Manuscript received June 15, 1932.

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Apparatus

The essential parts of the apparatus used in the experiments with the carbon dioxide in the liquid state are shown diagrammatically in Fig. 1. The Raman tube *D* consisted of a Pyrex tube, of length 17 cm., diameter 2.5 cm. and with 5-mm. walls, clamped between the brass top and base by means of three brass rods. Leather washers that had been soaked in hot wax were used at the joints to prevent leaks. The upper part of the brass top consisted of a removable brass part with a glass window cemented on its inner surface. A copper tube of small bore led from the top to a carbon dioxide cylinder. Directly opposite was an outlet tube, 5 mm. in diameter, attached to a gauge and a safety valve. A circular silvered mirror, *M*, was placed in a horizontal position on a raised central portion of the brass base of the Raman tube. The tube was immersed in methyl hydrate contained in the large Dewar flask, *A*.

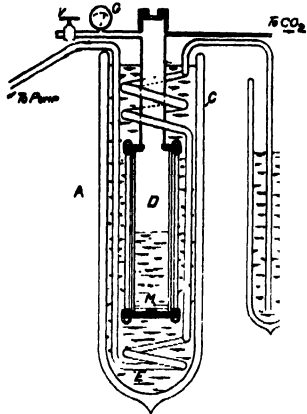


FIG. 1. Apparatus used in obtaining the Raman effect of clear liquid carbon dioxide.

It was necessary that any temperature between -50°C. and -100°C. be available during the course of the experiment. As the usual refrigerants are suitable only for a small range of temperature they could not be employed in this case. It was also impossible to use a freezing mixture containing solid carbon dioxide, due to the fact that such a mixture would permit but little light to pass through it from the source of incident radiation. A system that involved the use of liquid air as a cooling agent was finally employed. Copper tubing was fashioned into a coil which consisted of several turns about the upper part of the Raman tube and of two turns of the same diameter in the methyl hydrate below the base. The tubing which led to and away from the lower coil was placed as close as possible to the brass rods in order that a minimum of incident light might be prevented from reaching the light-scattering medium. One end of the coil was immersed in a flask, *B*, filled with liquid air, and the other end was connected to a rubber tube leading to a vacuum pump. The operation of the pump caused liquid air to be drawn up into the coils and the temperature of the methyl hydrate was decreased rapidly. As soon as the pump was stopped the temperature of the refrigerant increased. With this method of cooling it was possible to control the temperature accurately and when working at -60°C. the temperature could be changed at a rate of three degrees per minute. Pouring liquid air on the surface of the methyl hydrate lessened considerably the time taken for the first cooling from room temperature down to -60°C. , for the rate of cooling was then ten degrees per minute. The temperature of the methyl hydrate was read on a pentane thermometer.

In order to obtain clear liquid carbon dioxide it was necessary to overcome serious experimental difficulties since carbon dioxide does not exist in the liquid state at ordinary pressures. Solid carbon dioxide sublimates and the lowest pressure under which the liquid exists is 75 lb. per sq. in., and the temperature is -56°C . If the pressure be reduced below 75 lb. per sq. in. a white opaque snow forms which is unsuitable for scattering experiments. If the temperature be allowed to fall below -56°C . the liquid turns into a solid which remains clear for a time and then becomes clouded. Above -56°C . the pressure in the tube increases rapidly with temperature. The above shows the necessity for accurate and sensitive pressure and temperature control.

The procedure for obtaining the clear liquid carbon dioxide was as follows. The temperature of the methyl hydrate was lowered by the methods given above to -75°C . Then the pressure was increased slowly from one atmosphere and white solid carbon dioxide began to form on the mirror and inner surface of the Raman tube. The pressure was increased further to 75 lb. per sq. in., then, the temperature was increased until at -56°C . the solid changed to a clear colorless liquid.

The primary source of light was a special mercury lamp. It was constructed of Pyrex tubing of 2 cm. diameter in the form of a vertical helix, consisting of four turns 15 cm. in diameter. The arc, approximately eight feet in length, when surrounded by cylindrical polished aluminium reflectors gave a light of very great intensity along the axis of the helix. This axis coincided with that of the Raman tube during an exposure.

The lamp was operated on the 220-volt d.c. circuit and carried 6 amp. In order to start it, heat was applied by means of a large gas burner and the arc was struck by employing 50,000 volts from the 60-cycle a.c. circuit. After the lamp was started the cylindrical reflector was placed in position, and an electric fan was used to prevent overheating of the lamp and reflector.

The light scattered upwards along the axis of the Raman tube passed through the window at the top, and the mirror, *M*, reflected back along the axis the light scattered downwards. Suitable blackened metal diaphragms and blackened metal tubes cut down the amount of extraneous light reaching the slit of the spectrograph. Before making an exposure an image of the mirror *M* was focused by the lens *L* on the slit *S*. By this arrangement the light which arose from the walls of the Raman tube, and was not cut out by the diaphragms, was focused off the slit on all sides.

In order to lessen the continuous background and to isolate groups of lines in the spectrum of the incident light various filtering solutions were used. These were dissolved in the methyl hydrate.

A specially constructed direct vision spectrograph with a camera lens having a focal length of 8 cm. and providing a dispersion of about 70 \AA per mm. gave Raman spectrograms with exposures of $\frac{3}{4}$ to $1\frac{1}{2}$ hour's duration.

Ilford Golden Iso-zenith plates, *H* and *D* 1400, gave the best results for the region between $\lambda\ 4000\text{ \AA}$ and $\lambda\ 5000\text{ \AA}$.

The experimental arrangement described above proved very satisfactory for obtaining the Raman effect of the liquid, but attempts to use it for the solid were unsuccessful.

The solid was formed by reducing the temperature to -92°C . at the same time holding the pressure constant at 32 lb. per sq. in. The clear solid formed slowly, but grew inwards from the walls of the tube as well as upwards from the mirror, consequently a solid cylinder was not formed. The large amount of light arising from the surface of the solid on the walls caused fogging of the photographic plate after a short exposure.

In order to form a clear cylinder of the solid it was necessary to have the base of the Raman tube at a much lower temperature than that of the glass walls. This was effected by replacing the copper coils *E* by the part shown in Fig. 2. This consisted of inlet and outlet tubes *A* and *B* made of German silver, and a cylindrical container also of German silver but with a copper top that fitted snugly against the brass base of the Raman tube. Experiments made using this cooling device proved highly successful, for a cylindrical column of very clear solid carbon dioxide was formed, of which good Raman spectrograms were obtained with exposures ranging from $1-1\frac{1}{2}$ hours.

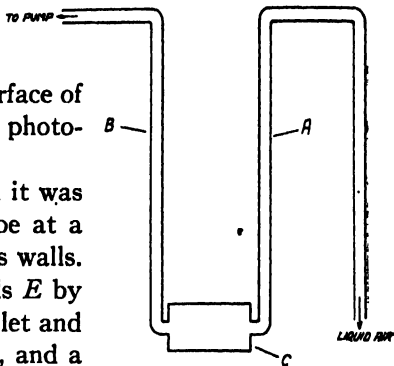


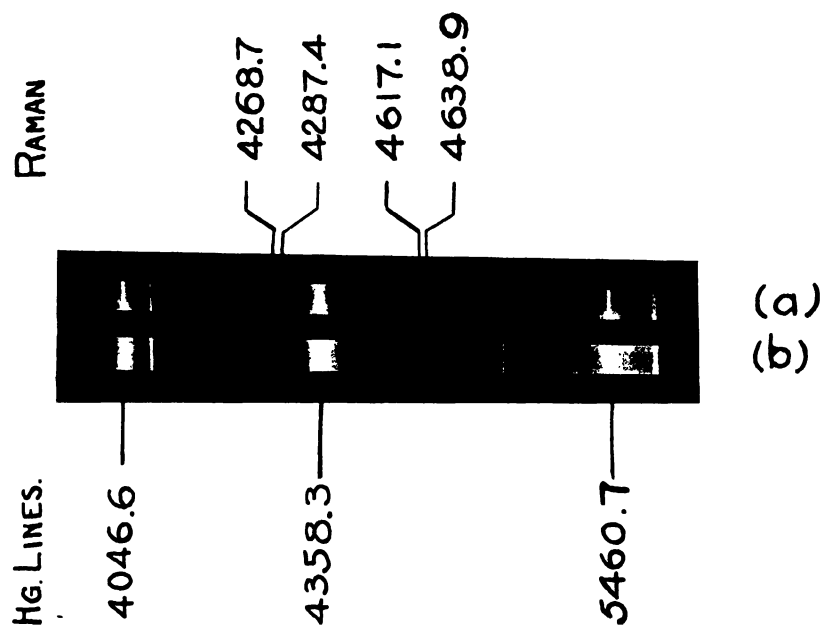
FIG. 2. Part used instead of the copper coils in experiments with clear solid carbon dioxide.

Experimental Results and Discussion

The Raman effect with carbon dioxide in the gaseous state has been investigated by a number of workers and four Raman lines have been observed in the spectrum of the scattered light. In the first column of Table I are given the values of the frequency shifts as obtained by Dickinson, Dillon and Rasetti. Two strong lines were observed by them with relative intensities 10 and 15. The stronger of the two was accompanied by a faint companion of longer wave-length whereas the other strong line was accompanied by a line of intensity 1 on its short wave-length side.

The spectrum of the light scattered by the liquid contained two sharp Raman lines with relative intensities of 5 and 15, while microphotometer tracings gave evidence of two other lines of lesser intensity corresponding to the weak components observed in the spectrum of the gas. The frequency shifts and intensities are shown in the second column of Table I. A reproduction of the spectrogram obtained is shown in Plate I-(a).

Bär (1), employing the powdered crystal method, failed to observe any Raman lines in the spectrum of the light scattered by carbon dioxide in the solid state. Spectrograms taken by us clearly exhibited the two stronger Raman lines. The relative intensities of those lines and their corresponding frequency shifts are given in the third column of Table I and a reproduction of one of the spectrograms is shown in Plate I-(b).



- (a). Raman spectrum of the light scattered by clear liquid carbon dioxide.
 (b). Raman spectrum of the light scattered by clear solid carbon dioxide.

It will be seen from Table I that the frequency shifts and intensities observed are practically identical for the gaseous, the liquid and the solid state. In this respect the case of the carbon dioxide molecule is analogous to that of other non-polar molecules.

These results also support the view that the carbon dioxide molecule undergoes no change in form throughout the temperature range -92°C. to 20°C.

TABLE I
FREQUENCY SHIFTS AND RELATIVE INTENSITIES FOR CARBON DIOXIDE

Gas		Liquid		Solid	
$\Delta\nu$ (cm. $^{-1}$)	Int.	$\Delta\nu$ (cm. $^{-1}$)	Int.	$\Delta\nu$ (cm. $^{-1}$)	Int.
1264.5	1	Faint line		—	
1285.1	10	1285.5	5	1285	5
1387.7	15	1387.5	15	1388	15
1408.4	1	Faint line		—	

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1. BÄR, R. *Nature*, 124: 692. 1929.
2. DICKINSON, R. G., DILLON, R. T. and RASETTI, F. *Phys. Rev* 34: 582-589. 1929.

APPLICATIONS SPECTROSCOPIQUES DE LA DECHARGE SANS ELECTRODES¹

PAR GEORGES DÉJARDIN²

Sommaire

L'étude spectroscopique de la décharge sans électrodes constitue l'un des meilleurs procédés de séparation des spectres correspondant aux différents degrés d'ionisation de l'atome. On utilise dans ce but la décharge annulaire obtenue avec des tubes cylindriques renfermant le gaz considéré sous faible pression, et l'on examine les modifications spectrales qui résultent de l'accroissement progressif de l'intensité d'excitation. Le classement empirique auquel on est conduit est en excellent accord avec les résultats basés sur les théories quantiques de l'émission. Les conclusions relatives aux gaz rares et au mercure font l'objet d'une discussion détaillée. Dans le cas du phosphore, la même technique a permis de répartir plusieurs centaines de raies dans plusieurs groupes distincts correspondant aux étapes successives de l'ionisation de l'atome.

Un gaz monoatomique pur traversé par une décharge électrique intense doit être considéré comme un mélange de plusieurs substances; celles-ci sont constituées par des atomes neutres et par des ions de charges variées, dont la concentration dépend des conditions expérimentales, telles que la pression, la tension et le mode d'excitation. En général, le rayonnement obtenu comprend donc plusieurs spectres indépendants qu'il s'agit de reconnaître et d'attribuer avec le maximum de probabilité aux divers centres d'émission. A cet égard, on ne peut obtenir de résultats certains qu'en faisant intervenir les arguments décisifs fondés sur la considération des séries spectrales, sur l'effet Zeeman, ou encore sur l'extension des lois des doublets des rayons X aux spectres optiques.

En réalité, il est toujours difficile, et même parfois impossible, d'utiliser directement les méthodes précédentes et de chercher, par tâtonnements, à identifier des séries ou des multiplets caractéristiques. Il faut donc s'adresser d'abord à un procédé pratique de séparation qui, mettant en jeu des intensités d'excitation graduellement croissantes, puisse permettre de différencier les raies d'un même élément et de les répartir dans divers groupes sensiblement homogènes. Le classement ainsi obtenu servira de point de départ pour la recherche des régularités spectrales, conformément aux lois rigoureuses qui régissent la structure des spectres.

Parmi les techniques qui conduisent à cette analyse expérimentale préalable des spectres, l'excitation des raies spectrales par choc électronique donne sans aucun doute les résultats les plus faciles à interpréter. Toutefois, l'émission d'un gaz soumis au bombardement électronique est en général très peu intense, et l'enregistrement photographique du spectre nécessite souvent de très longues durées de pose. La méthode du choc électronique est indispensable pour contrôler directement les prévisions de la théorie quantique des spectres et l'existence des niveaux d'énergie. Mais elle n'a été que très rarement utilisée pour différencier les spectres caractéristiques des divers degrés d'ionisation (17).

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Communication à la Société de Physique et de Chimie de Montréal (11 février 1932).

² *Professeur à l'Université de Lyon, Chargé d'un cours à l'Ecole Polytechnique de Montréal (février-mars 1932).*

L'étude spectroscopique de la décharge sans électrodes permet au contraire d'effectuer cette séparation d'une manière simple et rapide. Les avantages immédiats de ce mode d'excitation sont bien connus et ont été signalés par J. J. Thomson dès 1891. Les impuretés dégagées par les électrodes des tubes ordinaires sont évitées; la luminescence obtenue possède un éclat suffisant et les conditions d'excitation sont faciles à régler. Pour isoler commodément les spectres d'ordres successifs, on utilise le type de décharge "en anneau", facile à obtenir au moyen d'oscillations amorties. L'élément étudié est généralement introduit dans un tube cylindrique de silice fondue (par exemple: 12 cm. de longueur et 3 cm. de diamètre), fermé par des glaces planes et portant un enroulement inducteur d'une dizaine de spires. Le circuit de décharge, dont fait partie cet enroulement, comprend un éclateur formé de deux tiges métalliques dont l'écartement peut être modifié et réglé à volonté. On alimente ce circuit au moyen d'un transformateur relié d'autre part à un condensateur de capacité appropriée. Dans la plupart de nos expériences, la fréquence des oscillations correspondait à une longueur d'onde comprise entre 400 et 800 mètres.

Lorsque la pression est convenable, la décharge oscillante qui traverse l'enroulement illumine brillamment le gaz ou la vapeur. On projette alors sur la fente d'un spectrographe l'image de la luminosité qui se trouve localisée principalement dans un espace annulaire, au voisinage des spires du solénoïde. Lorsqu'on utilise un appareil stigmatique, les spectrogrammes obtenus présentent à la fois des raies longues traversant tout le champ, et des raies courtes émises par le bord de l'anneau. Les premières sont, par exemple, les raies d'arc et les autres celles du premier spectre d'étincelle. En agissant sur l'éclateur réglable, de manière à augmenter le potentiel de décharge, les raies primitivement longues s'atténuent, se concentrent parfois dans la région moyenne et tendent à disparaître. Au contraire, celles qui étaient courtes se renforcent et s'allongent peu à peu, tandis que de nouvelles raies courtes apparaissent sur les bords du champ. La comparaison des spectrogrammes correspondant à des tensions croissantes, basée sur l'intensité et la longueur des raies, permet donc de distinguer plusieurs groupes homogènes qui diffèrent entre eux de la même manière que le spectre d'arc diffère du premier spectre d'étincelle. L'étude des variations d'intensité et de l'évolution des raies suffit à la rigueur pour obtenir ce résultat. L'emploi d'un instrument stigmatique n'est donc pas absolument indispensable.

En 1923, L. et E. Bloch (5) ont proposé d'identifier provisoirement les groupes de raies ainsi séparés avec les spectres d'étincelle successifs, dont les centres d'émission sont les ions de charge simple ou multiple. Dans le cas du mercure, ils ont pu distinguer très nettement trois catégories de raies d'étincelle, désignés par E_1 , E_2 et E_3 , et appartenant vraisemblablement aux spectres Hg II, Hg III et Hg IV. Nous avons ensuite appliqué le même procédé aux gaz monoatomiques de l'atmosphère. D'autres corps, déjà très nombreux (métaux alcalins, cadmium, halogènes, soufre, sélénium, tellure, antimoine) ont été examinés dans les mêmes conditions au cours des dernières années.

Pour tous ces éléments, l'analyse expérimentale dont le principe vient d'être indiqué réussit très régulièrement.

Toutefois, il est impossible d'affirmer en toute rigueur que les groupes homogènes du spectre d'étincelle correspondent effectivement, pour chaque élément, aux divers degrés d'ionisation de l'atome. On ne peut justifier cette hypothèse qu'en s'adressant à des corps dont les différents spectres soient déjà partiellement identifiés, par application directe des lois spectroscopiques. Pour certains éléments étudiés par nous, les spectres d'étincelle ont été récemment l'objet d'une analyse théorique plus ou moins complète. Le moment semble donc venu de mettre en évidence la concordance remarquable de nos prévisions avec les résultats théoriques. Dans le cas des gaz rares, la classification empirique fournie par la décharge sans électrodes a réellement servi de guide dans la recherche des régularités spectrales (24). Les critiques qui ont été présentées à ce sujet (53, 54) sont complètement injustifiées.

Gaz rares

(a) Néon

L'emploi de la décharge sans électrodes nous a permis en 1926 (7) de découvrir et de caractériser avec certitude deux spectres d'étincelle du néon. Ce résultat a été obtenu en utilisant plusieurs tubes renfermant du néon rigoureusement pur sous une pression variant de quelques centièmes de mm. à un mm. de mercure. Lorsque la pression est relativement forte on distingue seulement sur les spectrogrammes les raies d'arc et celles du premier groupe de raies d'étincelle, considéré par nous comme représentant le spectre Ne II. La Fig. 1 représente une série de quatre spectrogrammes obtenus dans ces conditions et correspondant à des excitations d'intensité croissante. Elle montre que, pour un certain réglage de l'éclateur du circuit oscillant, les raies d'étincelle courtes apparaissent, parmi les raies longues Ne I, à partir de 4800 Å. Nous avons retrouvé sur nos clichés la plupart des raies Ne I observées et classées par Paschen (42, 43). Quelques raies d'étincelle très intenses sont souvent présentes dans le spectre du néon fourni par la décharge ordinaire non condensée. En particulier, les trois raies 3713, 3694 et 3664 Å. figurent parmi les raies observées dans l'arc par Paschen, sans être cependant rattachées à aucune série du spectre Ne I. La Fig. 2 montre précisément que ces trois raies se différencient nettement par leur longueur et leur aspect des raies d'arc voisines. Les deux raies 3694 et 3664 sont les plus intenses du multiplet 'P' 'P' qui a servi de point de départ pour la recherche des régularités dans le premier spectre d'étincelle du néon.

Le spectre d'étincelle d'excitation supérieure, que nous attribuons à l'atome doublement ionisé, ne se développe dans la décharge sans électrodes qu'aux très faibles pressions. Les raies les plus intenses et les plus caractéristiques se trouvent entre 2800 et 2500 Å. Sur les spectrogrammes de la Fig. 3, on reconnaît aisément les deux groupes de raies d'étincelle. Pour les plus faibles pressions, toutes les raies sont longues et le critère de séparation repose sur les différences d'évolution constatées à tension croissante.

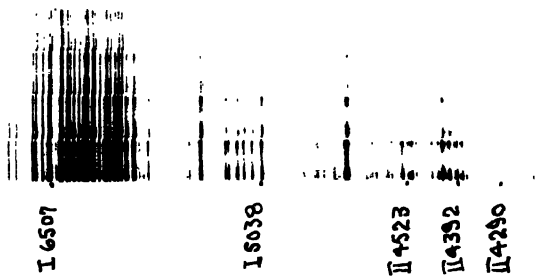


Fig.1. Ne I et Ne II.

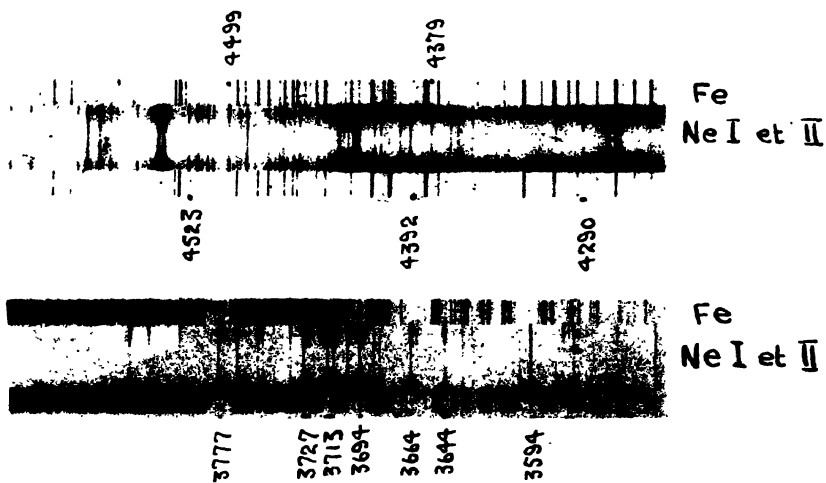


Fig.2.

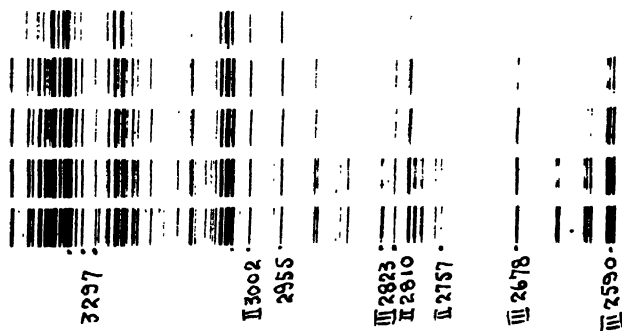


Fig.3. Ne II et Ne III.

La structure du spectre Ne II (doublets et quadruplets) a été analysée par Kichlu (26, 27) et surtout par de Bruin (11, 12, 15, 48). La classification proposée est entièrement d'accord avec nos prévisions. Parmi les 264 raies classées par de Bruin, 250 avaient été attribuées par nous à l'atome simplement ionisé. D'autre part, les analogies signalées par le même auteur entre les spectres Ne III et F II ne font intervenir que des raies appartenant effectivement au second groupe d'excitation élevée, séparé avec une grande précision du spectre Ne II.

(b) *Argon*

La classification en séries du spectre A I a été largement développée par Meissner (37, 38, 39). Dans l'intervalle 7067–3034 Å, sur 579 raies classées du spectre A I, 34 seulement figurent dans notre liste de raies d'arc publiée en 1924 (6). Elles comprennent d'ailleurs les raies les plus intenses observées par Meissner. Cela prouve seulement que les conditions d'excitation utilisées dans notre travail (pression: 0.3 mm.) ne sont pas favorables à l'émission du spectre d'arc. En effet, la très grande majorité des raies classées A I ne se retrouvent nullement dans les trois groupes de raies d'étincelle séparés au moyen de la décharge sans électrodes et désignés par E_1 , E_2 et E_3 . Le désaccord ne porte que sur une vingtaine de raies tout au plus et nous estimons qu'il provient, dans la plupart des cas, d'une interprétation défectueuse de nos spectrogrammes de faible dispersion. En outre, parmi les raies attribuées à un spectre d'étincelle et classées cependant par Meissner dans le spectre A I se trouvent trois raies (4865.91–3564.27–3388.35) classées également par de Bruin (4, 13, 14) dans le spectre A II. Enfin, les listes de Meissner comprennent encore 152 raies non classées, parmi lesquelles 16 sont visibles sur nos clichés et se rattachent, d'après notre classification empirique, au groupe E_1 (spectre A II).

Les doublets et quadruplets de A II ont été reconnus par de Bruin (4, 13, 14) et par Rosenthal (47). La classification obtenue porte sur 238 raies, parmi lesquelles 73 raies peu intenses n'ont pas été observées par nous (6). Toutes les autres, à l'exception d'une seule (3480.49) se trouvent rangées effectivement dans le premier groupe E_1 de raies d'étincelle. Les deux spectres d'ordre supérieur E_2 et E_3 séparés grâce à la décharge sans électrodes n'ont été jusqu'ici l'objet d'aucune tentative de classification.

(c) *Krypton*

Les recherches théoriques récentes s'étendent sur les quatre spectres Kr I, Kr II, Kr III et Kr IV. La comparaison avec les prévisions expérimentales présente donc pour ce gaz un intérêt particulier. Le spectre d'arc Kr I a été analysé par Meggers, de Bruin et Humphreys (34, 36). Dans l'intervalle 6905–3184 Å, les auteurs précédents indiquent 275 raies classées, dont 33 raies parmi les plus intenses ont été en effet observées par nous (6) comme raies d'arc. Mais les autres raies Kr I de Meggers, de Bruin et Humphreys ne figurent pas, en général, dans les autres groupes de raies d'étincelle E_1 , E_2 et E_3 . Comme pour l'argon, le désaccord apparent porte sur un petit nombre de cas plus ou moins douteux faisant intervenir des groupes de raies très voisines ou

des coïncidences probables de raies appartenant à des spectres différents. Dans nos expériences de 1924, nous avons seulement en vue la séparation des divers spectres d'étincelle, et les conditions requises pour l'émission de l'ensemble du spectre d'arc (forte pression, tension faible avec longue durée de pose) n'ont pas été réalisées.

La structure des spectres d'étincelle du krypton a été partiellement analysée par Kichlu (28) (Kr II), de Bruin (3) (Kr II) et Acharya (1, 2) (Kr III et IV). A un très petit nombre d'exceptions près, toutes les raies classées dans le spectre Kr II par Kichlu (147 raies, entre 6905 et 2960 Å) et par de Bruin (91 raies) font partie du premier groupe E_1 de raies d'étincelle. L'accord entre la théorie et les attributions provisoires basées sur la décharge sans électrodes peut être considéré comme parfait.

Le Tableau I donne la répartition des raies des groupes E_2 et E_3 dans les deux spectres Kr III et Kr IV, d'après Acharya:

TABLEAU I

	Kr II	Kr III	Kr IV	Raies obs. par déch. sans élec. et non classées
E_2		62	5	68
E_3	3	34	11	146

Si les régularités indiquées par Acharya sont réelles, la séparation de Kr III et de Kr IV n'a pas été effectuée d'une manière absolument correcte. Toutefois, cette séparation est fondée ici sur l'examen des spectrogrammes fournis par un seul tube renfermant du krypton sous une pression de 0.6 mm. Nous estimons qu'une discrimination beaucoup plus précise aurait été obtenue en se plaçant systématiquement dans d'autres conditions, comme pour Ne II et III. Dans les spectres E_1 , E_2 et E_3 , le nombre des raies observées et non classées est considérable, ce qui montre à quel point les classifications proposées jusqu'ici sont incomplètes.

(d) Xénon

La structure du spectre X I est maintenant bien connue d'après le travail expérimental et théorique de Meggers, de Bruin et Humphreys (35). Entre 9163 et 3343 Å, 261 raies ont été classées, parmi lesquelles 178 se trouvent effectivement dans le spectre d'arc de la décharge sans électrodes (6, 21). Le tube utilisé par nous renfermait du xénon pur sous une pression de 1 mm. de mercure, et le spectre d'arc obtenu ainsi est beaucoup plus complet que pour les gaz précédents. Les autres raies classées X I, à quatre exceptions près, ne font pas partie des groupes de raies d'étincelle E_1 , E_2 et E_3 . Parmi les raies non classées de la liste de Meggers, de Bruin et Humphreys, deux appartiennent à l'arc d'après leur évolution à tension croissante: 8258.0 et 6728.00 Å, mais quatre se trouvent rangées dans le groupe E_1 (8143.8, 6277.2, 6114.7 et 4215.5).

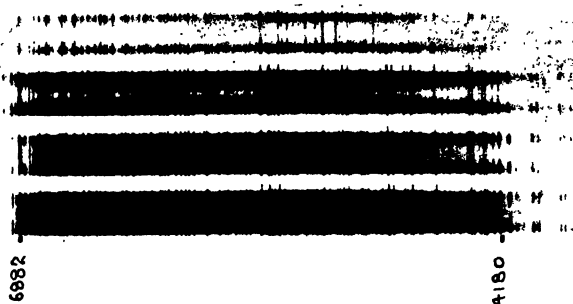


Fig. 4. Xénon I à IV.

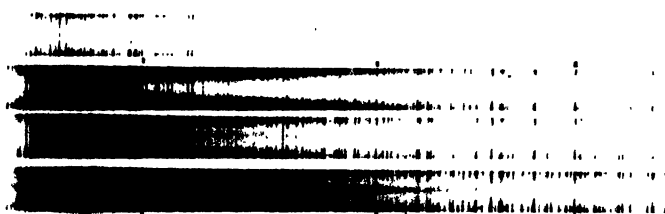


Fig. 5. Xénon I à IV.

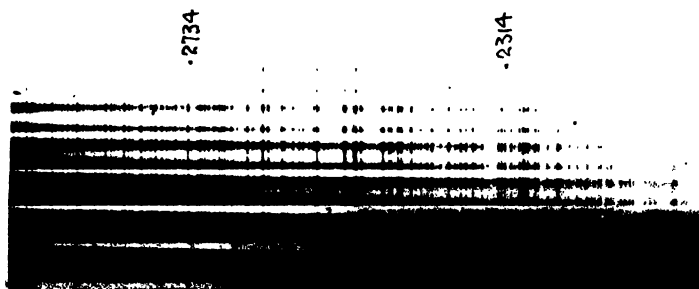


Fig. 6 Xénon II à IV.

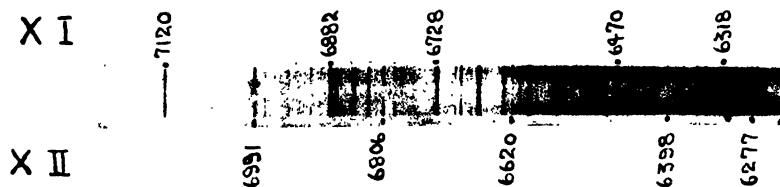


Fig. 7.

Les doublets et quadruplets de X II ont été aussi identifiés partiellement par Humphreys, de Bruin et Meggers (24), en excellent accord avec nos prévisions de 1924 et 1930 (6, 21). Sur 130 combinaisons indiquées, 80 raies sont placées dans le groupe E₁, et les autres sont invisibles sur nos spectrogrammes. La classification de X II est encore très incomplète, car nous avons observé jusqu'à présent près de 1000 raies constituant le groupe homogène E₁ du xénon (9000–1850 Å) (6, 20, 21).

A titre d'exemples, les Figs. 4 à 6 représentent des spectrogrammes du xénon fournis par la décharge sans électrodes, et ayant servi à la séparation des spectres E₁, E₂ et E₃. Les spectres de raies sont accompagnées d'un spectre continu intense localisé dans la région annulaire de grand éclat, au voisinage de l'enroulement. On observe alors deux bandes symétriques dont l'intensité décroît à partir des bords vers le centre du champ. En outre, les excitations très intenses donnent naissance à un spectre continu émis par la région centrale du tube, et visible sur les clichés sous la forme d'un trait axial.

Le spectre de la Fig. 7 a été obtenu avec un réseau concave de 150 cm. de rayon. Avec le montage au minimum de déviation, l'astigmatisme est suffisamment réduit pour qu'on puisse différencier nettement par leur longueur les raies X II des raies X I.

Mercure

La classification du premier spectre d'étincelle du mercure Hg II (doublets et quadruplets) a été établie par Paschen (44) et par Naudé (41). Les termes de doublets ont été obtenus en prenant pour point de départ les attributions proposées par Carroll (16) et confirmées par divers résultats expérimentaux (18). En raison de l'extrême complexité du spectre d'étincelle global du mercure, l'analyse théorique de Hg II ne pouvait être tentée qu'après l'avoir séparé expérimentalement des spectres d'ordre supérieur Hg III et IV. A cet égard, la technique utilisée par Paschen et Naudé (étude de la lueur négative d'un tube à décharge spécialement construit, et influence de l'hélium sur les intensités relatives des raies), est certainement beaucoup moins efficace que la décharge sans électrodes, employée dans le même but par L. et E. Bloch (5), G. Déjardin (18) et Laffay (30). Parmi les nombreuses raies non classées des tables de Paschen et de Naudé, on trouve en effet environ 130 raies appartenant aux groupes E₂ et E₃, et qui, par conséquent, auraient été immédiatement exclues par la technique du tube sans électrodes. L'accord est en général satisfaisant entre la classification empirique obtenue par cette méthode et les résultats théoriques. Toutefois, les combinaisons indiquées par Naudé comprennent une vingtaine de raies qui, d'après leur évolution (étudiée par la décharge sans électrodes) appartiennent probablement aux spectres Hg III et Hg IV. Pour les raies 3312.31 Å et 1994.72 Å, classées par Paschen et Naudé, le potentiel d'excitation, mesuré directement, est nettement supérieur à 40 volts, ce qui semble incompatible avec leur attribution à Hg II. La réalité de certaines combinaisons n'est donc pas suffisamment justifiée.

La classification de Paschen-Naudé a été récemment critiquée et révisée par McLennan, McLay et Crawford (33). Dans ce nouveau travail, la plupart

des raies attribuées à Hg II font effectivement parti du groupe E_1 . Toutefois, la raie $\lambda = 2234 \text{ \AA}$ se rattache par son évolution au groupe E_2 (Hg III), comme on peut s'en assurer en examinant les spectrogrammes de la Fig. 8, sur lesquels la raie incriminée se trouve immédiatement à gauche de Hg IV 2232 \AA . Le potentiel d'excitation de cette raie est d'ailleurs supérieur à 40 volts (22).

Le groupe E_2 , séparé par la décharge sans électrodes—et identifié provisoirement avec Hg III—comprend trois raies (2354, 2244 et 2214) qui trouvent effectivement leur place dans l'esquisse de classification du spectre Hg III proposée par McLennan, McLay et Crawford (32).

La Fig. 8 montre avec quelle netteté s'effectue la séparation des raies d'étincelle du mercure en trois groupes E_1 , E_2 et E_3 (Hg II, III et IV). On distingue parfaitement sur cette figure les trois raies Hg III classées par McLennan, McLay et Crawford. Les tables classiques ne donnent qu'une description assez imparfaite du spectre d'étincelle globale du mercure. La décharge sans électrodes constitue sans aucun doute un excellent procédé d'excitation permettant la révision complète des données expérimentales relatives à ce spectre.

En examinant le cas du xénon, nous avons fait remarquer que l'émission de la décharge sans électrodes comprend, en plus des spectres de raies, un spectre continu intense dont les caractères dépendent de la pression et du régime de la décharge. Les spectres continus analogues obtenus avec le mercure ont été étudiés systématiquement par Robertson, McKinnon et Zinn (46), et par Volklinger (52). L'émission continue de la vapeur de mercure très raréfiée soumise à une excitation très énergique (Fig. 8) diffère profondément de celle que l'on observe avec le même tube à haute température, ou avec les lampes à mercure d'usage courant fonctionnant sous un régime assez poussé (18).

Phosphore

Les raies fondamentales des différents spectres du phosphore (de P I à P V) ont été découvertes par application directe des règles spectroscopiques. Il était donc possible, pour cet élément, de soumettre à un excellent contrôle le procédé d'analyse empirique fondé sur la décharge sans électrodes. Les résultats obtenus en 1927 n'ont pas été publiés intégralement (19). Nous les décrirons ici avec quelques détails, et nous montrerons en même temps les conditions d'application de la méthode dans un cas particulier.

Une petite quantité de phosphore a été introduite par distillation dans un tube cylindrique de silice fondue, après élimination des gaz occlus par chauffage prolongé à 900°C . Le tube a été ensuite scellé sous un excellent vide et intercalé dans le circuit de décharge, après mise en place de l'enroulement inducteur. La décharge lumineuse ne s'amorce que lorsque la pression de la vapeur de phosphore est suffisante. Pour qu'il en soit ainsi, il est nécessaire de chauffer assez fortement le tube, et on y parvient en le plaçant complètement dans un four approprié. Toutefois, il est indispensable d'empêcher toute condensation sur la fenêtre destinée au passage du rayonnement, ce qui oblige à maintenir constamment cette fenêtre à une température un peu plus élevée. Pour cette raison, l'emploi d'un four s'est révélé très incommode, et on s'est généralement contenté de chauffer énergiquement le tube avec un chalumeau

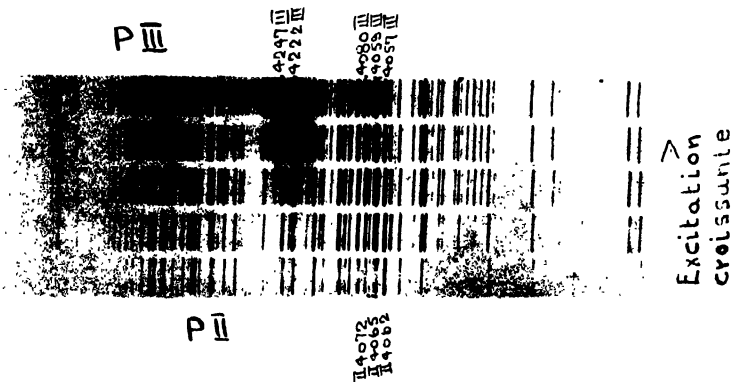
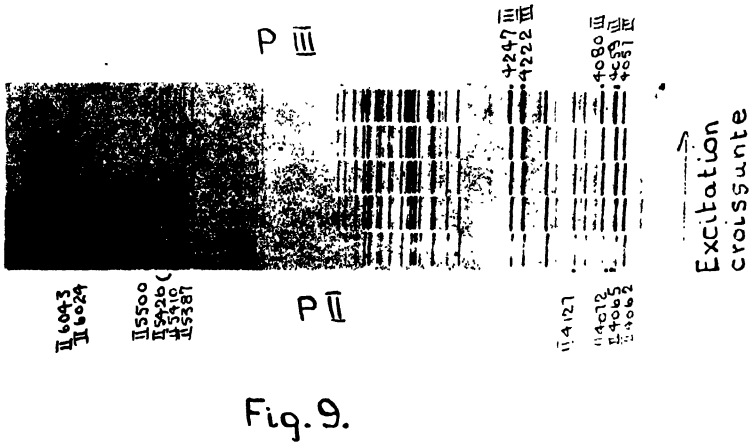
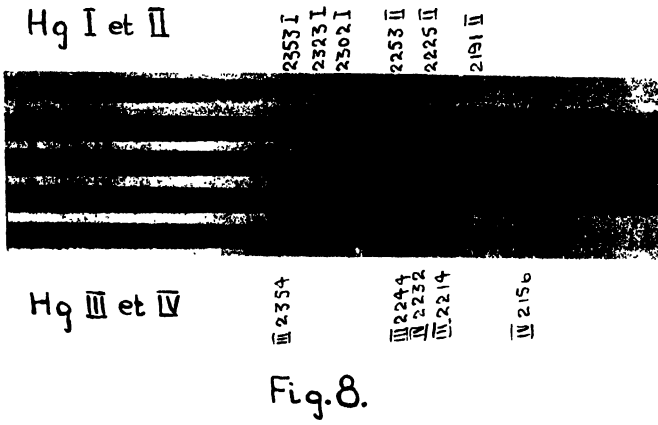


TABLEAU II

Int.	λ	Etincelle	Obs.	Int.	λ	Etincelle	Obs.
5	6043.05	1	S., PIII S.	0	4609.46	1?	G.
4	24.14	1	S., PIII S.	0	08.04	2?	G.
0	5588.25	1	G., PII B.	1	05.52	1	G.
0	83.33	1	G., PII B.	8	01.96	1	G.
0	41.18	1	G., PII B.	3	4595.98	1	G.
0	07.13	1	G., PII B.	8	95.53	1	G.
4	5499.71	1	G., PII B.	8	89.78	1	G.
1	83.55	1	G., PII B.	8	87.90	1	G.
3	60.85	1	G.	4	81.76	1	G.
4	50.65	1	G., PII B.	2	74.90	2	G.
1	37.28	1	G.	5	65.21	1	G.
8	28.10	1	G.	2	61.93	2	G.
5	25.92	1	G., PII B.	6	58.03	1	G., PII B.
5	09.65	1	G., PII B.	6	54.80	1	G., PII B.
5	5386.87	1	G., PII B.	2	48.40	2	G.
1	78.11	1	G., PII B.	3	46.03	2	G.
6	45.81	1	G.	3	44.97	2	G.
5	44.72	1	G., PII B.	2	41.12	3?	G.
5	16.07	1	G., PII B.	1	40.20	1	G.
5	5296.09	1	G., PII B.	1	33.81	1	G., PII B.
6	93.63	1	G.	6	30.78	1	G., PII B.
1	53.48	1	G.	1	25.06	2?	G.
1	03.85	2	G.	5	22.93	2	G.
3	5191.40	1	G., PII B.	2	19.96	1	G.
1	52.20	1	G., PII B.	4	07.55	2	G.
1	41.49	1	G.	3	04.06	2	G.
1	5097	2	G.	2	01.2	2	G.
0	51.59	1?	G.	7	4499.17	1	G.
2	40.74	1	G.	1	87.41	1?	G.
3	4969.64	1	G., PII B.	3	85.29	2	G.
2	54.32	1	G., PII B.	4	83.66	1	G., PII B.
4	43.41	1	G., PII B.	6	79.74	2	G.
2	35.55	1	G.	7	75.26	1	G., PII B.
2	27.16	1	G., PII B.	5	67.97	1	G., PII B.
0	4888.52	1?	G.	3	66.10	1	G., PII B.
0	83.65	1?	G.	7	63.70	1	G.
2	76.98	2	G.	6	62.94	1	G., PII B.
1	72.73	1	G.	6	52.44	1	G.
3	64.38	1	G., PII B.	3	43.87	2	G.
1	54.69	1	G.	5	28.15	2	G.
0	44.25?	1?	G.	4	23.9	1	G.
0	23.84	1?	G., PII B.	6	23.55	1	G.
0	19.34?	1?	G.	6	20.64	1	G.
1	14.2	1?	G.	4	17.30	1	G., PII B.
1	05.96	1?	G.	5	14.60	1	G.
2d	4792.06	1?	G.	5	14.28	1	G., PII B.
1	78.80	1?	G.	1	11.94	1	G.
1	64.84	1?	G.	1	01.96	1	G., PII B.
1	63.91	1?	G.	3	00.99	2	G.
2	55.79	2	G.	6	4385.33	1	G.
3	39.49	1	G.	1	79.62?	2?	G.
0	35.88?	1?	G.	1	64.09	1?	G.
0	26.84	2?	G.	1	47.89	2?	G.
4	24.25	2	G.	2	16.84	2	G.
2	20.26	1	G.	1	07.94	2	G.
0	17.00	1?	G.	Id	4294.11	1? 2?	G.
3	00.79	1	G.	4	88.52	1	G.
1	4698.56	1	G.	2	84.29	2	G.
5	78.94	1	G., PII B.	2	82.81?	2	G.
3	75.78	2	G.	1	71.85	2	G.
0	61.78	1?	G.	3	49.57	2	G., PIII M.B.
6	58.11	1	G.	7	46.68	2	G.
2	41.72	1	G.	4	44.55	1	G.
3	28.70	1	G., PII B.	1	33.96	2	G.
5	26.60	1	G.	4	30.10	1	G.
2	22.70	1	G.	3	24.43	1	G.
3	13.8?	1?	G.	8	22.15	2	G., PIII M.B.
	12.83	2?	G.	1	16.56?	1?	G.

TABLEAU II—*suit*.

Int.	λ	Etincelle	Obs.	Int.	λ	Etincelle	Obs.
2	4202.24	2	G.	1	3668.59	2?	G.
3	4193.42	1	G.	4	64.19	1	G.
3	89.08	2	G.	1	59.26	1?	G.
2	88.07	2	G.	5	53.38	1	G.
8	78.36	1	G.	3	31.30	1	G.
3	66.73	1	G.	3	23.10	1	G.
4	60.56	1	G.	3	20.65	1	G.
	59.75	1	G.	6	17.09	1	G.
2	57.33	2	G.	1	3587.35	2	G.
2	52.4	2	G.	1	83.60	2	G.
1	51.19?	2	G.	1	80.35	2	G.
4	43.84	2	G.	2	77.60	2	G.
4	30.77	1	G., PII B.	2	70.33	1?	G.
6	27.49	1	G., PII B.	4	66.42	1	G., PIII S.
3	20.78	1	G., PII B.	3	62.47	1	G.
2	18.96	1	G.	3	59.92	1	G., PIII S.
4	17.09	1	G., PII B.	4	56.48	1	G.
6	09.19	1	G.	5	52.48	1	G.
0	02.1	2?	G.		51.15		G.
4	4094.43	1	G.	2	36.29	1?	G.
4	91.53	1	G., PII B.	4	33.66	1	G.
3	89.25	2	G.		33.06	1	G.
8	80.04	2	G., PIII M.B.	4	30.24	1	G.
5	72.13	1	G., PII B.	3	27.10	1	G.
4	64.64	1	G., PII B.		19.22	1	G.
4	62.08	1	G., PII B.	3	18.60	1	G., PII B.
7	59.27	2	G., PIII M.B.	5	16.15	1	G.
4	57.39	2	G., PIII M.B.	7	07.36	1	G.
6	44.49	1	G.	5	04.50	1	G.
4d	36.22	1	G., PII B.	5	02.99	1	G., PII B.
4	33.68	1	G., PII B.		3490.44		G.
6	19.45	1	G.	6	88.77	1?	G.
5	3997.16	2	G., PIII B.	2	85.00	2	G.
2	85.86	2	G.	5	78.73		G., PII B.
8	78.27	2	G.		77.44	1	G.
2	73.10?	1	G.		74.14		G.
3	67.54	2	G.	6	72.87	1	G., PII B.
6	57.62	2	G., PIII B.		70.82		G.
5	51.50	2	G., PIII B.		26.19		G., PII B.
4	33.37	2	G., PIII B.	7	24.87	1	G., PII B.
1	28.57	2?	G.	6	19.24	1	G., PII B.
2	27.29	1?	G.	0	13.51	3?	G.
3	22.71	2	G., PIII B.		06.93		G.
4	14.26	2	G.	6	04.33	1	G.
5	04.78	2	G., PIII B.	4	3395.35	2?	G.
4	3895.02	2	G., PIII B.	6	78.76	1	G.
6	85.17	1	G.		77.52		G.
3	77.69	2	G.	2	72.70	2?	G.
0	37.14	2?	G.	2	71.10	3	G., PIV B.M.
7	27.44	1	G.	4	64.43	3	G., PIV B.M.
5	02.07	2	G., PIII B.	2	60.49	2?	G.
1	3799.4	1	G.	5	47.71	3	G., PIV B.M.
2	95.09	1	G., PII B.	0	41.40?	1?	G.
2	93.60	1	G., PII B.	4	18.24	1	G.
1	88.06	1	G.	7	08.85	1	G.
0	86.69	1	G., PII B.	1	3296.30	2?	G., PIII S.
1	75.02	2?	G.	3	83.20	2	G.
4	68.70	1	G., PII B.	2	80.20	2	G.
2	61.81	1	G., PII B.	3	77.80	2	G.
3	44.21	2	G., PIII B.	2	59.19	1	G.
3	28.66	1	G.	2	53.58	2	G.
2	23.62	1	G.	8	33.61	2	G., PIII M.B.
5	17.62	1	G., PIII B.	8	19.30	2	G., PIII M.B.
4	17.02	1	G.	2	08.50	2	G.
3	15.85	1	G., PII B.		03.4		G.
2	10.45	1	G.	3	02.57	2?	G.
7	06.05	1	G.		01.80		G.
6	3676.26	1	G.				

TABLEAU II—suite

Int.	λ	Arc	Etincelle	Obs.	Int.	λ	Arc	Etincelle	Obs.
1	3194.7		1?	G.	5	2739.90		3	G., PIV B.M.
4	86.24		2	G.		39.32			G., PIII B., PIV B.M.
4	84.82		2	G.					
0	76.6		2?	G.	4	29.19		3	G., PIV B.M.
0	75.14		2?	G.		28.80			G., PIV B.M.
4	71.84		2	G.	2	24.86		3	G., PIV B.M.
3	63.87		2	G.	0	18.14		3?	Q.
3	62.34		2	G.	2	15.80		2	G.
2	57.73		2?	G.	0	13.71		3?	Q.
1d	54.74		2?	G.	4	10.40		2?	G.
	53.23			G.	1	08.06	1		Q.
1	51.67		2?	G.	1	06.12		2	Q.
3dr	44.38		2?	G.	1	2696.30		2	G.
3	30.30		2?	G.	1	94.07		2	G.
1	24.30		1?	G.	1	88.06	A		G., PI K.
1	11.5		2?	G.	1	86.58		2	G.(1)
4	3051.9	1		G.	2	84.98		2	G. PIII B.
3d	38.2		3?	G.	2	83.59		1	Q.
	28.7			G.	6	80.36		2	G.
5	27.2		1	G.	3	78.83		1	Q.
0	21.52		1?	G.	2	77.22	A		G., PI K.
0	2991.2		2?	G.	2	76.28		2?	G.
2	80.6		2?		0	68.90		1?	Q.
2	77.6		2?	G.	4	63.99		2	G., PIII B.
1	63.96		2?	G.	5	60.39		1	Q.
1	58.7		2?	G.	1	58.85		1?	Q.
3dr	48.14	1		G.	0	57.45		1?	Q.
1	45.52		2?	G.	0	50.84		1?	Q.
2	39.67	1		G.	3	49.88		1	Q.
1	37.36		2?	G.	5	44.20		1?	3?
1	27.43		2?	G.	4	38.18		1	G., PII B.
2	24.08		2?	G.	6	36.77		1	G., PII B.
4	18.56			G.	7	32.62		2	G.
2	10.39		2	G.	4	28.54		1	G., PII B.
8	2895.32		2	G., PIII M.B.	6	25.15		1	G., PII B.
1	87.29		2?	G., PIII B.	3	24.76		1	G., PII B.
5	83.90		2	G., PIII M.B.	2	18.31		1	Q.
3	82.75		2?	G., PIII B.	2	15.16		1	Q.
3	77.53		2	G., PIII B.	6	11.05		2	G.
3	75.9	1		G.	7	06.02		1	G., PII B.
1	73.16		2	G., PIII B.		05.45			G.
5	66.14		2	G., PIII B.	3	03.72		1	G., PII B.
3	62.06		2	G., PIII B.	2	2599.00		1	Q.
2	56.96		2	G., PIII B.	1	94.73		1	Q.
4	26.5		1	G.	0	93.81		1?	Q.
2	25.16		1	G.	0	86.57		1?	Q.
4	22.99		1	G.	2	68.25		1?	Q.
0	15.86		1?	G.	1	62.89		1?	Q.
0	09.48		1?	G.	6	54.96	A		G., PI L.L.
2	2789.99		2?	G., PIII B.	7	53.32	A		G., PI L.L.
4	80.79		3?	G., PIII B.	0	47.81		1?	G.
1	71.83		1?	G., PIII B.	2	42.84		1	Q.
4	58.52		3?	G., PIII B.	7	35.63	A		G., PI L.L.
3	52.71		2	G., PIII B.	7	34.01	A		G., PI L.L.
					1	31.82		1	Q.

(1) Cette raie d'excitation élevée coïncide pratiquement avec une raie d'arc visible sur les spectres de moindre excitation. Celle-ci correspond évidemment à la combinaison des deux termes $3p^2P_{0,1}^0$ et $4s^4P_{0,1}$ indiqués par Kiess.

TABLEAU II—*suite*

Int.	λ	Arc	Etincelle	Obs.	Int.	λ	Arc	Etincelle	Obs.
5	2500.92		1	G., PII B.	3	2289.19		1	Q.
6	2497.33		1	G., PII B.	5	86.37		1	Q.
4	95.95		1	G., PII B.	6	84.85		1	Q.
7	84.15		1	G., PII B.	5	80.91		1	Q.
5	81.96		1	G., PII B.	1	77.66		1	Q.
1	80.55		1	Q.	1	74.85		1	Q.
1	78.22		3?	G.	0	72.64		1?	Q.
0	77.77		1?	G.	4	66.21		1	Q.
0	62.6		1?	G.	0	57.98		1?	Q.
1	57.62		1	Q.	0	47.30		1?	Q.
2	54.47		1	G., PII B.	1	46.76		1?	Q.
1	52.99		1	Q.	2	42.39	A		Q. PI K.(1)
0	51.51		1?	Q.	2	39.06		3?	Q.
0	49.16		1?	Q.	2d	35.48	A		Q., PI K.
5	27.75		2	G., PIII M.B.	1	34.51	A		Q., PI K.
6	19.73		2	G., PIII M.B.	1	28.32		3?	Q.
0	2398.88		1?	Q.	1	27.42		3?	Q.
1	97.11		1	Q.	1	23.11			Q. PI K.
1	93.25		1	Q.	1	10.21		1	Q.
2	89.85		1	G.	0	2195.19		1?	Q.
1	88.88		1	Q.	3	54.09	A		S., PI L.L.
2	67.06		2	Q.	2	52.95	A		S., PI L.L.
2	66.12		2	Q.	4	49.13	A		S., PI L.L.
1	45.47		1?	Q.	3	36.11	A		S., PI L.L.
3	33.80		1	Q.	2	35.42	A		S., PI L.L.
1	31.42		1?	Q.	1	07.60		2?	Q.
1	30.33		1?	Q.	2	04.33		2?	Q.
0	28.74		1?	Q.	1	2099.22		2?	Q.
2	27.48		1	Q.	3	98.61		2?	Q.
1	25.47		1?	Q.	4	93.66		2?	Q.
2	23.13		1	Q.	1	91.28		2?	Q.
3	21.63		1	Q.	0	90.34		2?	Q.
1	18.62		1?	Q.	0	89.89			Q.
3	16.87		1	Q.	0	88.47		2?	Q.
4	14.61		1	Q.	1	84.23		2?	Q.
1	06.29		2?	Q.	2	33.36	A		S., PI L.L.
0	02.42		2?	Q.	1	32.32	A		S., PI L.L.
1	00.99		2?	Q.	1	24.32	A		S., PI L.L.
4	2298.25		1	Q.	2	23.32	A		S., PI L.L.

(1) Cette raie ne figure pas dans la liste de raies P I publiée par Kiess, mais elle correspond évidemment à la combinaison des deux termes $3p^2D_{14}^0$ et $4s^4P_{014}$.

à gaz. Il est possible, par ce procédé, de réaliser une constance suffisante de la température au cours d'une série de poses spectrographiques (durée totale de l'ordre de l'heure), pour différents régimes de la décharge.

Pour les plus faibles excitations, correspondant à une distance des tiges de l'éclateur égale à 3 mm., la luminosité a l'aspect d'un étroit anneau blanchâtre au voisinage de la paroi de quartz. Cet anneau gagne progressivement sur le centre obscur, dont il est parfois séparé par une bordure jaunâtre. Lorsque la tension est plus élevée (coupure de 6 à 8 mm.), on distingue nettement trois régions constituées par le centre obscur de plus en plus réduit, un anneau intermédiaire de couleur jaune citron correspondant aux raies P II situées dans l'orangé, et un anneau extérieur blanc bleuâtre dans lequel prédominent les raies P III des régions bleue et violette. Avec les plus fortes excitations

le groupe (Arc, E_1 , E_2 et E_3) correspondant à son aspect et à son évolution à tension croissante. La dernière colonne contient encore, s'il y a lieu, l'indication du spectre P I, P II, P III ou P IV dans lequel la raie a été effectivement classée par Bowen (B.) (8, 9), Millikan et Bowen (M.B. ou B.M.) (10, 40), Miss Saltmarsh (S.) (49, 50), McLennan et McLay (L.L.) (31) ou Kiess (K.) (29).

Dans le Tableau III ont été réunies les raies visibles sur les spectrogrammes et ne faisant pas partie des listes publiées antérieurement. Il est probable que la plupart d'entre elles appartiennent réellement au phosphore. Celles qui se rattachent nettement aux groupes E_1 (P II) ou E_2 (P III) sont indiquées par un ou deux astérisques. Les longueurs d'onde de ces raies nouvelles ont été déterminées, à quelques dixièmes d'angström près, par comparaison avec les raies voisines. Un certain nombre de raies doivent être cependant attribuées à l'arsenic, au soufre, à l'oxygène et peut-être au sélénium.

Le Tableau IV donne les longueurs d'onde de quelques raies nouvelles (à quelques dixièmes d'angström près) observées dans la partie la moins réfrangible du spectre visible. Elles appartiennent très probablement au phosphore et se classent toutes dans le groupe E_2 (P II).

TABLEAU IV

Int.	1	2	3	1	1	2	2	1	3
λ	6507.9	6503.4	6460.1	6435.5	6367.0	6165.2	6087.4	6055.2	6033.9

Pour le spectre d'arc et les divers spectres d'étincelle, les observations sont en accord presque rigoureux avec les attributions prévues par McLennan et McLay, ou Kiess (P I), par Millikan et Bowen (P II à P V) et par Bowen (P III). Dans l'intervalle spectral examiné, les raies séries des deux premiers spectres d'étincelle définissent deux groupes homogènes nettement séparés, dans lesquels viennent se ranger un très grand nombre de raies supplémentaires. Ces dernières présentent exactement les mêmes caractères d'évolution que les raies séries; elles doivent être attribuées aux mêmes centres d'émission P^+ et P^{2+} .

Les triplets caractéristiques de P IV n'apparaissent qu'avec les plus puissantes excitations réalisées. Quant aux raies attribuées par Millikan et Bowen au spectre P V (atome "dépouillé"), elles restent invisibles sur tous les spectrogrammes (à l'exception d'une seule: 3175.14). Cette absence est certainement due à l'insuffisance des intensités mises en jeu avec l'installation employée (transformateur et condensateur).

Les spectrogrammes des Fig. 9 et 10 donnent une idée de la précision avec laquelle la séparation des divers groupes de raies a été effectuée. Les raies dont la longueur d'onde est indiquée en regard sur les clichés font partie des régularités découvertes par Millikan et Bowen. En particulier, on distingue nettement trois raies P III intenses et d'évolution tout-à-fait caractéristique: 4247, 4222 et 4080 Å. Ces trois raies ont été décelées récemment par Struve (38) dans les spectres des étoiles chaudes des premières subdivisions de la

classe B (par exemple, 88 γ Pegasi), qui présentent également les raies caractéristiques de Al²⁺, Si²⁺ et S²⁺.

En résumé, la technique utilisée conduit effectivement à la séparation des spectres caractéristiques des différents degrés d'ionisation de l'atome de phosphore. Les tableaux précédents peuvent servir de point de départ pour le développement des classifications ébauchées par Millikan et Bowen.

Les exemples précédents suffisent pour montrer l'intérêt du procédé d'analyse expérimentale basée sur l'étude spectroscopique de la décharge sans électrodes. La même technique peut fournir des indications sur l'adsorption des ions par la paroi du tube (durcissement progressif des tubes à gaz rares) et sur les réactions chimiques qui se produisent dans certains cas (formation de silicium réduit et libération d'oxygène, décomposition des chlorures). Il y aurait intérêt à étudier systématiquement ces phénomènes avec des gaz différents et des verres de compositions variées. Au cours de nos expériences sur l'hélium et le néon, nous avons relevé sur les spectrogrammes un très grand nombre de raies du silicium et de l'oxygène, dont la présence résulte d'une désintégration appréciable des parois des tubes. L'examen d'une centaine de raies du silicium nous a même permis de retrouver très exactement les spectres Si I, Si II, Si III et Si IV, en classant les raies d'après leurs caractères distinctifs d'évolution.

Le mécanisme de la décharge sans électrodes ne semble pas encore complètement élucidé. Il est probable qu'une étude plus poussée de l'émission des différentes régions du tube, et notamment des variations d'intensité de certaines raies photographiées avec un appareil rigoureusement stigmatique, apporterait une contribution importante à la connaissance de la répartition du champ de haute fréquence qui produit l'ionisation du gaz.

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THE EFFECT OF DIASTATIC MALT UPON THE LOAF VOLUMES OF COMMERCIAL FLOURS¹

BY R. H. HARRIS²

Abstract

A series of ten commercial flours of different protein contents and baking strengths was baked by a variety of methods to determine the effect of adding to the dough progressively increasing quantities of diastatic malt. The two lower malt concentrations, 2 and 4% respectively, increased the resulting loaf volumes in every case, but the highest percentage of malt, 6%, increased the loaf volume over the values obtained with 2 and 4% of malt only when the highest protein flours were used. Flours which yielded unsatisfactory loaves when baked with the simple formula gave much better results when malt was added.

The inclusion of 0.002% of potassium bromate increased the loaf volumes except in the cases of two flours evidently of low diastatic power, where little improvement was shown on the results with the simple formula. The further addition of 2% of malt reacted very favorably with the higher protein members of the series, but had no effect upon these two flours.

The addition of 2 and 4% of sucrose to the simple formula did not prove as beneficial as the addition of like quantities of diastatic malt.

No significant relation was found between protein content and loaf volume in the absence of malt and bromate.

Introduction

In the evaluation of baking strength different investigators have had recourse to the use of diastatic malt in the baking formula. The principal virtue of this ingredient appears to lie in its ability to render available a constant supply of fermentable carbohydrate for the yeast enzymes during the period of fermentation. Flours low in diastatic power may produce loaves of satisfactory volume when baked with diastatic malt, provided the protein content is sufficient and that the flour is in good condition. Malt is largely used in commercial bakeries, but in this case the flavor imparted to the bread may be a contributing factor. There is a tendency toward progressive darkening of the loaf with increasing quantities of malt, associated with a loosening up of the texture and coarsening of the grain.

Geddes, Larmour and Malloch (3), when determining the baking value of western Canadian wheats, employed a formula containing diastatic malt and stressed the value of this method when flours of poor gas-producing power are used. A high response to malt indicates that the gas production is not sufficient to make full use of the gas-retaining capacity of the dough, while a low response indicates that the gas production is already adequate or that gas retention is poor. These workers (3) used a concentration of 1% of diastatic malt.

The investigations of Herman and Hart (7) have also demonstrated the stimulative effect of diastatic malt resulting in increased loaf volumes. Harris (5) found that four flours of widely different protein contents yielded higher

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loaf volumes when baked with both potassium bromate and diastatic malt than when the bromate was used alone. Diastatic malt extract was also used by Jørgensen (8) for the purpose of differentiating between baking strength and diastatic activity. The concentration of the extract was only 0.15%, but the yeast content, 0.7%, would make a relatively small demand upon the fermentable sugar in the dough.

The danger of yeast starvation in fermenting doughs was discussed by Harris (6) in reference to baking with cooked potato. He found that while no starvation was apparent with 3% of yeast and 2.5% of sucrose in the flours examined, the presence of potato required the use of diastatic malt if the best results were to be obtained. The use of malt also permitted the use of higher yeast concentrations without detrimental effects. The malt concentration was 2%.

In consideration of the value of diastatic malt in baking, the writer decided to investigate the effect on baking strength of increasing dosages of this ingredient superimposed upon the regular baking formula of flour, sugar, salt, water and yeast.

Materials and Methods

A series of commercial flours of various types was available for the study. These flours were produced from western Canadian hard spring wheat, contained a fairly wide range of crude protein and ash content, and should be quite representative of Canadian hard wheat commercial flour. Descriptions of these flours with crude protein and ash content are presented in Table I.

TABLE I
DESCRIPTION, CRUDE PROTEIN AND ASH CONTENT OF FLOURS

Flour No.	Description	Crude protein, %	Ash, %
		(Calc'd to 13.5% moisture basis)	
61	Unbleached middlings flour	11.2	.31
62	First patent flour, claimed unsatisfactory for baking	12.0	.38
63	Small mill straight (long extraction)	12.0	.48
64	First patent	12.3	.34
65	Baker's patent	13.3	.46
66	Baker's patent	13.4	.51
67	Baker's patent milled from high protein wheat blend	15.8	.51
68	Evidently a "stuffed"* flour	16.1	.94
69	First clear flour	16.9	.76
70	Strong break flour (unbleached)	17.4	.46

* The crude protein and ash contents are increased by mixing in various quantities of clear flour with the patent; this of course also lowers the cost of production.

The methods used in baking the flours were (a) the simple or basic procedure advocated by Blish (1), and (b) various modifications consisting of the addition of other ingredients (improvers) to the basic method, as described by

the writer in previous publications (4, 5). These methods will not be discussed in detail here. The malt was added, in each case, from a graduated cylinder, using a mixture of malt and water, 4 cc. of the fluid containing 1 gm. of malt. The potassium bromate was introduced by means of a pipette, using a stock solution, 1 gm. of bromate to 1 litre of distilled water. The crude protein and ash were determined according to standard methods used by the American Association of Cereal Chemists.

Three concentrations of malt were used, 2, 4, and 6%. While these last two dosages would doubtless be considered very high, they were included to determine the effect of increasing the malt to the highest possible concentration without causing detrimental effects. Two series of bakings were also made with an additional 2 and 4% of sucrose respectively, for purposes of comparison with the malt bakings. Bakings were likewise made with potassium bromate and 2% of malt, and also bromate without malt.

Results

The loaf volumes obtained from the ten flours baked by the different methods are shown in Table II. These results are calculated on a moisture basis of 13.5%. Baking scores were not computed, principally because of the intense darkening caused by the higher concentrations of malt, which would have materially lowered the score. It also seemed more pertinent to determine the effect of the malt concentrations upon loaf volume than upon other properties of the loaf.

TABLE II
LOAF VOLUMES OBTAINED USING THE VARIOUS BAKING FORMULAS (13.5% MOISTURE BASIS)

Flour No.	Loaf volumes, cc.								Protein, %
	Simple	Malt			0.002% KBrO ₃		Sucrose		
		2%	4%	6%	2% Malt	No malt	2%	4%	
61	520	565	568	535	500	508	530	550	11.2
62	444	525	552	503	494	494	475	523	12.0
63	446	495	520	506	485	437	422	446	12.0
64	545	557	585	555	535	535	555	566	12.3
65	580	650	680	630	680	640	590	612	13.3
66	577	596	616	576	646	675	596	680	13.4
67	605	615	645	625	760	780	665	682	15.8
68	460	503	550	565	560	560	513	533	16.1
69	556	600	620	650	735	650	570	560	16.9
70	648	720	740	810	945	780	620	700	17.4
Average	538	583	608	595	634	606	554	585	

Examining first the volumes obtained with the simple formula, there appears to be no regular tendency toward increase in loaf volume with increasing protein content. Flours Nos. 62, 63, and 68 when baked by this method yielded very small loaves, while the strong break flour No. 70 gave an exceptionally large loaf. No. 67 also produced a very good loaf without the addition of a flour improver.

When the results obtained with diastatic malt are considered, there is evident a general increase in loaf volume over the values yielded by the simple formula. The three flours which previously gave low volumes yielded values quite similar to those obtained with the other samples. A concentration of 2% of malt increased the volume in every instance over the values from the simple formula, while 4% of malt enhanced this effect. The final treatment with 6% of malt appeared to exert a harmful influence, and for all except the last three higher-protein samples there is shown a decrease in volume below the values obtained with 4% of malt. This would seem to indicate that with exceptionally high protein flours of this type it is possible to add larger quantities of diastatic malt than are ordinarily used, with the production of loaves of increased volume. In the case of even the weakest flours of the series, the results yielded with 6% of malt are higher than the values with the simple formula. Apparently, when malt is superimposed on the basic formula it exerts an influence similar to other flour improvers, causing an increase in loaf volume, the degree of increase or response depending largely upon the protein content of the flour. If the malt concentration rises above a certain limit a falling-off in volume is evident, which is again very similar to the effect of other improvers. It was found with all the flours examined in this study, that the addition of 4% of malt to the simple formula produced no injurious effects.

The bakings with potassium bromate yielded results very similar to those obtained with the addition of malt, with the exception of Nos. 62 and 63. These flours did not respond as favorably to bromate as to malt. The series containing 2% of malt in addition to the bromate showed higher volumes with the last two higher-protein flours. With flour No. 70 especially it was possible to use larger quantities of the malt. Sucrose did not appear to increase the loaf volume as much as equal concentrations of malt when added to the simple formula. These findings are reflected in the average loaf volume shown at the foot of the table.

The mean loaf volumes obtained with all the flours by the eight different baking methods are shown in Table III. No. 63 gave the lowest value of those shown in this table, and seemed quite unsatisfactory for baking purposes. No. 62 was next in order, and would be considered rather poor in baking

TABLE III
MEAN LOAF VOLUME BY EIGHT BAKING METHODS

Flour No.	Loaf volume, cc.	Protein, %	Flour No.	Loaf volume, cc.	Protein, %
61	534	11.2	66	620	13.4
62	501	12.0	67	672	15.8
63	470	12.0	68	531	16.1
64	554	12.3	69	618	16.9
65	633	13.3	70	745	17.4

strength. The strong break flour, No. 70, showed the highest loaf volume; No. 68 gave low results, due probably to "stuffing" with lower quality flour.

The mean responses to the different improvers were calculated and are shown in Table IV. These data were then arranged in groups on the basis of protein content. The samples containing from 11.2 to 13.2% of crude protein were placed in the first group, those with 13.2 to 15.2% in the second, those with 15.2 to 17.2% in the third, and the last in the group over 17.2%.

The average responses for each group were then computed. The data thus arranged are given in Table V, and shown graphically in Fig. 1. This figure exhibits a striking similarity between the effect of the various concentrations of malt; the 2 and 4% treatments are approximately parallel throughout, each showing a tendency to rise at the final concentration of 17.4%. The baking data obtained with 6% of malt reveal a sharper response with flours with more than 13.5% of protein, with a very decided upward trend to the 17.4% protein content. The larger response is shown in the treatment with 0.002% bromate and 2% of malt, the increase being practically a linear relation up to 16.5% of protein, where the line makes a very sharp upward rise. The response with bromate alone shows a gradual increase with increasing protein, but the line tends to level off at the final protein content. The responses to the addition of sucrose did not reveal any decided increase with increasing protein, but indeed show a slight tendency to decrease with higher protein content. The bakings with 2% of sucrose, especially at the higher protein concentration, appear out of line with the other results. Fig. 1 however shows that there is a general tendency toward increase in response with higher concentrations of malt, especially when the higher protein flours are considered.

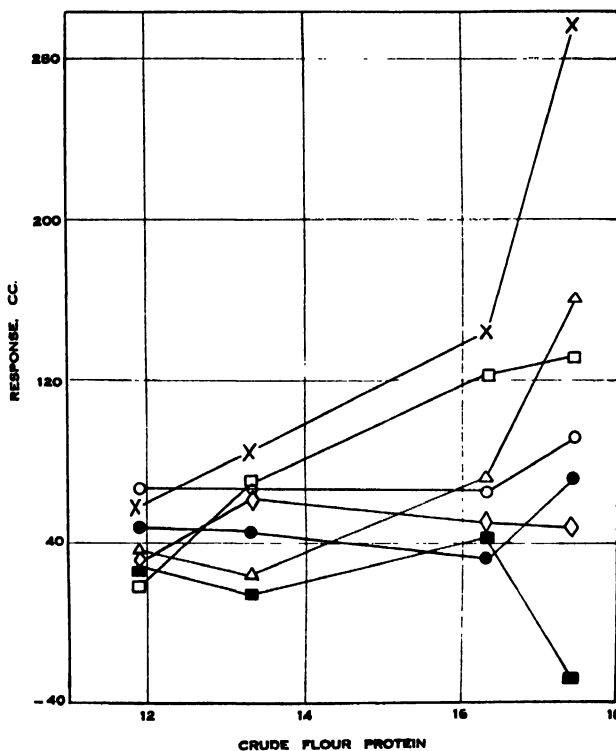


FIG. 1. Mean responses in loaf volume to different baking tests. Legend: ●, 2% malt; ○, 4% malt; △, 6% malt; X, bromate and malt; □, bromate; ■, 2% sucrose; ◇, 4% sucrose.

TABLE IV
RESPONSE TO MALT, BROMATE AND MALT, AND SUCROSE

Flour No.	Response, cc.							Protein, %
	2% Malt	4% Malt	6% Malt	0.002% Bromate,		2% Sucrose	4% Sucrose	
				+ 2% Malt	No malt			
61	45	48	15	−20	−12	10	30	11.2
62	81	108	59	50	50	31	79	12.0
63	49	74	60	39	−9	−24	0	12.0
64	12	40	10	−10	−10	10	21	12.3
65	70	100	50	100	60	10	32	13.3
66	19	39	−1	69	97	19	103	13.4
67	10	40	20	155	175	60	77	15.8
68	43	90	105	100	100	53	73	16.1
69	44	64	94	179	94	14	4	16.9
70	72	92	162	297	132	−28	52	17.4

TABLE V
AVERAGE PROTEIN CONTENT AND AVERAGE RESPONSES OF FLOURS, ARRANGED IN GROUPS ON THE BASIS OF PROTEIN CONTENT

Flour protein		Average response, cc.						
Range %	Average %	2% Malt	4% Malt	6% Malt	0.002% KBrO ₃		2% Sucrose	4% Sucrose
					+ 2% Malt	No malt		
11-13	11.9	47	67	36	59	19	27	32
13-15	13.3	44	69	24	84	68	14	67
15-17	16.3	32	65	73	145	123	42	51
17-19	17.4	72	92	162	297	132	-28	52

Discussion of Correlation Coefficients

The correlation coefficients computed between crude flour protein and loaf volume are shown in Table VI. The significance of these correlations may be judged by comparison with the points of minimum significance according to the number of pairs of observations. These values have been tabulated by Fisher (2). There are 10 pairs of values involved and the 5 and 1% points are respectively .632 and .765.

The coefficients calculated from baking methods which did not include malt or bromate in the formula are not significant. The presence of flours low in diastatic activity doubtless lowers the relation between protein and loaf volume in the absence of malt. Hence, it would appear advisable to include diastatic malt among the baking ingredients when baking a series of flours which might contain samples of poor diastatic power.

TABLE VI
CORRELATION COEFFICIENTS COMPUTED BETWEEN FLOUR PROTEIN AND LOAF VOLUME

Baking formula	Correlation coefficient
Simple	+ .483
Simple plus 2% diastatic malt	+ .690
Simple plus 4% diastatic malt	+ .564
Simple plus 6% diastatic malt	+ .763
Simple plus 0.002% potassium bromate	+ .745
Simple plus 0.002% potassium bromate plus 2% diastatic malt	+ .804
Simple plus 2% sucrose	+ .537
Simple plus 4% sucrose	+ .489
Value of r at 5% point	+ .632
Value of r at 1% point	+ .765

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"SMUTTY" WHEAT CAUSED BY *USTILAGO UTRICULOSA* ON DOCK-LEAVED PERSICARY¹

BY O. S. AAMODT² AND J. G. MALLOCH³

Abstract

Ustilago utriculosa (Nees) is reported for the first time in Canada on pale or dock-leaved persicary. It is a loose smut, readily dispersed when passing through a threshing machine.

Infected persicary plants growing in a wheat field were harvested with the grain, and in the threshing operation the grain was mechanically contaminated with smut spores. The grain was visibly covered with smut, but there was no odor as is the case when wheat is infested with bunt spores.

Wheat contaminated with persicary loose smut spores seems to be subject to the usual additional cost in handling common to "smutty" wheat caused by bunt. The effect of persicary smut on the loaf color was similar to that of bunt. These observations afford an example of the indirect ways in which weeds may cause losses in crop production.

In September, 1931, a sample of Garnet wheat was received from Chipman, Alberta, at the Western Grain Inspection Division Office in Edmonton. This sample was dark in color owing to the presence of what appeared to the eye to be numerous smut spores in the crease and brush of the kernels. The sample did not contain bunt balls and the characteristic odor of wheat infected with bunt, or stinking smut, (*Tilletia tritici* and *T. levis*) was absent. The sample was submitted to the senior writer by Mr. S. Thompson of the Grain Inspection Office for a more detailed examination.

The contents of the crease and brush and of the kernels were examined under the microscope and found to consist of numerous smut spores. These smut spores were similar to *T. tritici*, the organism producing wheat bunt, in the reticulations of the spore surface, but they were only one-half to two-thirds the usual size of bunt spores. The difference in the size of the spores, and the absence of the characteristic odor and of bunt balls, made it appear likely that the spores had come from some extraneous source.

This suspicion was strengthened by the fact that Garnet wheat possesses a high degree of resistance to bunt, and the presence of a sufficient number of spores to be visible to the naked eye has been rare. In testing spring wheats for reaction to bunt, Garnet showed the least infection of all the wheat varieties commonly grown in western Canada (1). In field surveys Garnet was observed to be relatively free from bunt. During the winter of 1930-31, 125 random samples of wheat grading "smutty" were obtained from the Government Grain Inspection Office at Edmonton. Only seven of these were Garnet, as determined by a growing test the following summer. Approximately 40% of the cars graded that year at the Edmonton office were Garnet,

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but only 5.5% of these grading "smutty" were of the Garnet variety. In the growing test of the 125 samples the following summer Garnet averaged less than 1% bunt, Marquis 19% and Red Bobs 39%. It seemed desirable in view of these facts to undertake the definite identification of the spores on the sample of Garnet originating at Chipman.

Samples of this grain contained numerous seeds of pale or dock-leaved persicary (sometimes called smart-weed or lady's thumb), *Polygonum lapathifolium* L., wild buckwheat, *Polygonum convolvulus* L., and lamb's quarters, *Chenopodium album* L. (Fig. 1, D).

The weed seeds were examined carefully and found to be sound and without any indication of having been infected with smut. After a second car of wheat from the same growers was graded smutty, a sheaf of grain was obtained, from the field where the grain was produced, containing a large percentage of weeds, especially persicary (Fig. 1, A). Approximately one-half of the persicary plants were heavily infected with a smut (Fig. 1, B). The inflorescence of the diseased plants consisted of smut balls, instead of seed, which under a very slight pressure broke and produced a dusky violet mass of powdery spores (Fig. 1, C). The smut was determined as *Ustilago utriculosa* (Nees) Tul. on *Polygonum lapathifolium* L. commonly known as the pale or dock-leaved persicary. The host is a weed found scattered across Canada. The identification of the smut was confirmed by Mr. I. L. Connors, Division of Botany, Ottawa, Canada. In a recent letter he stated that he had examined specimens of this smut on *Polygonum pennsylvanicum* L. from Fredericton, New Brunswick, in 1931, and that the smut had also been reported this year on *Polygonum persicaria* L. from Queen's County, Prince Edward Island, Canada. The smut is widely distributed in the United States on the sub-genus *Persicaria*, to which the above hosts belong. It is also reported from South America, Europe, Asia, and Australia.

The spores of *U. utriculosa* from persicary were identical with those on the kernels from the wheat grown in the same field. In the threshing the seed cases containing spores would be broken readily and their contents mixed with the threshed grain. The wind pressure from the blower would force the spores into the creases and brushes of the wheat kernels, from which they would not be easily dislodged.

While a microscopic examination failed to show any spores which were identical with bunt spores a further check on their possible presence in the sample was made by growing the grain under optimum conditions for bunt infection. This was done by placing the germinating seed in a refrigerator with a temperature of 10°C. which is the optimum temperature for infection with *T. tritici*. Previous experiments with bunt of wheat, not yet published, indicated that the number of bunted heads are much greater in resistant varieties when sown in the fall, when the days are short and the plant growth slow, than in the spring or summer, when the days are longer and the plant growth more rapid. In this experiment the seed was sown in November in the greenhouse, consequently the infection percentage on Garnet was high and not representative of its reaction to bunt when spring sown. Both species

of *Tilletia* found commonly in western Canada were used as artificial inoculum. Kota, a variety of spring wheat which is very susceptible to bunt, was included as a check. The results are given in Table I.

TABLE I
BUNT INFECTION OF GARNET INFESTED WITH *Ustilago utriculosa* AND GARNET AND KOTA ARTIFICIALLY INOCULATED WITH *Tilletia tritici* AND *T. levis*

Variety and sample	Inoculum	Number of plants		
		Total	Bunted	% Bunted
Garnet from Car 40 80 81	Seed naturally infested with <i>Ustilago utriculosa</i>	88	0	0
Garnet from Car 32 12 58	Seed naturally infested with <i>Ustilago utriculosa</i>	90	0	0
Garnet from Car 40 80 81 and 32 12 58	Seed naturally infested with <i>Ustilago utriculosa</i> and artificially inoculated with <i>Tilletia levis</i>	84	75	89
Garnet from Car 40 80 81 and 32 12 58	Seed naturally infested with <i>Ustilago utriculosa</i> and artificially inoculated with <i>Tilletia tritici</i>	80	70	88
Kota	Seed artificially inoculated with <i>Tilletia tritici</i>	59	51	86
Kota	Seed artificially inoculated with <i>Tilletia levis</i>	55	44	80

In the above experiment the high percentages of bunted plants of Garnet and Kota, when inoculated with spores of *T. tritici* and *T. levis*, indicate that optimum conditions prevailed for successful infection. Under these conditions the Garnet samples infested with *U. utriculosa* gave no indication of having *Tilletia* spores present, since not a single plant became bunted. An examination of the sheaves of grain from the infested field failed to show any bunted wheat heads.

Even though it was definitely demonstrated that the smut on the threshed grain was not bunt, the question still remained as to whether it was detrimental from the standpoint of milling and baking quality. The problem was unique and there appeared to be no precedent upon which an opinion might have been based. Through the kindness of Mr. W. H. Boyle, of the Alberta Wheat Pool, larger samples were obtained for milling and baking tests at the University of Alberta. It was unfortunate that a smut-free sample from the same field could not be obtained. No control sample was, therefore, available.

It was felt, however, that comparison with normal and artificially smutted samples of Garnet would give an indication as to whether contamination with persicary smut is detrimental to milling and baking quality. Accordingly, the following samples were tested:



A—Sprig of persicary (*Polygonum lapathifolium*) normal.

B—Sprig of persicary (*Polygonum lapathifolium*) infected.

C—Powdery spore mass of *Ustilago utriculosa*.

D—Garnet wheat heavily smutted with spores of *Ustilago utriculosa* and containing a mixture of persicary seeds.

No. 1. A sample of clean Garnet grown on the experimental plots at the University.

No. 2. Similar to sample No. 1, but artificially contaminated with bunt spores.

No. 3. Garnet from Chipman infested with persicary smut.

No. 4. Similar to No. 3 except that grain was artificially contaminated with bunt spores.

The two samples of Garnet from Edmonton naturally are not entirely comparable with those from Chipman since the two fields are approximately 40 miles apart. Climate and soil conditions, however, are similar in the two regions. The grain from both places was similar in vitreousness, plumpness and grade.

Previous to milling, all four samples were cleaned in a "clipper" fanning mill. All weed seeds and other foreign material were removed. They were then run through a "Eureka" horizontal wheat scourer. The grain was so thoroughly scoured that practically all of the brush was removed as well as a considerable portion of the bran layers. After this treatment the kernels were examined under the microscope. The grain in samples Nos. 2, 3 and 4 still contained a large number of spores in the crease and on the brush end. In sample No. 4 spores of both the persicary smut and wheat bunt were present in approximately equal numbers. These results indicate that in milling practice a thorough washing would be necessary in addition to scouring in order to remove the smut spores.

After scouring, the samples were milled and baked in order to note the effect of the smuts on the loaf volumes, texture and color. Simple and bromate tests were run on each sample. The loaf volumes are given in Table II.

TABLE II
LOAF VOLUMES OF SAMPLES OF GARNET WHEAT WHEN INFESTED WITH
PERSICARY SMUT AND WHEAT BUNT SPORES

Sample	Origin	Treatment	Loaf volume in cc.	
			Simple bake	Bromate bake
1	Edmonton	Clean	529	623
2	Edmonton	Bunt	540	661
3	Chipman	Persicary smut	511	548
4	Chipman	Persicary smut and bunt	494	514

The baking tests were run in duplicate and show the usual variability in loaf volume. The differences are not significant, with the possible exception of sample No. 4 which was contaminated with both smuts. There is a slight reduction in loaf volume. There were no differences in the color of the crust or shape of loaf. No odor was perceptible in the loaves baked from flour containing bunt.

There was a slightly grayish tinge to the loaves baked from samples Nos. 2 and 3 and a more marked one in sample No. 4 which contained both smuts.

All the samples were markedly yellow. Had the basic color of the flour been white the grey tinge would have been much more noticeable. The important fact is that the color of the sample contaminated with persicary smut was practically identical with that of the sample artificially contaminated with bunt. It is probable that the wheat in question would have to be treated in the same manner as bunted wheat before a satisfactory flour could be made from it.

Discussion

Weeds are recognized as being responsible for some of the greatest difficulties of crop production. Their control is one of the oldest and most expensive operations in agriculture. Weeds are injurious in many ways, the most important being the direct competition with the crop plants. This results in a decrease in crop yields and an increase in the cost of production. More indirect effects are the harboring of insects and diseases by weeds and the decrease in the land values. They also are the cause of certain diseases in man and animals.

One effect of weeds on crop production not often mentioned is the effect on quality of the crop. This is usually brought about indirectly, through competition for nutrients, moisture and sunlight. When the weeds are diseased they may serve as important sources of inoculum for crop plants. Even though the organism causing the disease does not attack the crop plant it may have a very detrimental effect on the quality of the crop and its market value. Loose smut of the dock-leaved persicary when present in a wheat field is an excellent example of this type of damage from weeds. The average loss per car of wheat which grades "smutty" is approximately fifty dollars. While the loss caused by the competition between the wheat plants and the weeds in the field is a greater one than that caused by the smut, yet the loss from smut is a very specific one which can be readily appreciated. It is one more good reason for weed control.

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A NON-SPECIFIC COMPLEMENT FIXATION REACTION DUE TO ION ANTAGONISM¹

BY A. HAMBLETON²

Abstract

The complement fixation test for tuberculosis gives a non-specific or falsely positive reaction if a balanced physiological saline, akin to Tyrode's solution, replaces the plain saline used in the test. The balanced saline must contain both calcium and magnesium in appropriate concentration. The non-specific reaction is given most strongly by Petroff's whole bacillus antigen, and not at all by "fat-free" tubercle bacillus antigens. The main features of the reaction, and its relation to antagonistic effects of sodium and calcium ions, are indicated.

There exists an extensive literature having reference to non-specific complement fixation reactions, and the false interpretation of these reactions still constitutes a limitation to the serological diagnosis of disease. Concerning the nature of the substances which produce the non-specific reaction, little is known, although the mechanism of the reaction appears to be similar to that in the specific complement fixation reactions. In each case the antigen is sensitized, *i.e.*, some serum component so modifies the antigen that complement is adsorbed. For example, in the non-specific complement fixation reactions caused by certain bacteria and normal serum, Mackie and Finkelstein (8) have pointed out that "the complement fixing agent in the serum is 'adsorbed' at 0° C. by bacteria, which became 'sensitized' in this way".

The non-specific reaction described in this paper is distinct from those to which the writer has referred above in that only traces of inorganic salts are used to confer on the antigen the ability to fix complement. The essential features of this new non-specific reaction will be described here, and the mechanism of the reaction and its bearing upon immune reactions in general will be indicated in a second paper (5).

Part I. Experimental

Origin of these Experiments. The non-specific reaction indicated above was first observed in a protein free sample which had been dialyzed against flowing tap water. This sample gave a clear positive reaction in the tuberculo-complement fixation test (hereinafter denoted as T.C.F. test), whereas similar material dialyzed against distilled water gave a negative reaction. In neither case was the sample anticomplementary.

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Contribution from the Mara Laboratories at the Queen Alexandra Sanatorium, London, Ontario, Canada, with financial assistance from the National Research Council of Canada. A report upon an early stage of this work was included in a thesis for the degree of Master of Arts in the Department of Biochemistry, University of Toronto.

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Trial showed that the tap water alone*, on being made isotonic with sodium chloride, gave a positive T.C.F. test, and that similar results could be obtained by using an appropriate and quite dilute solution of calcium chloride and magnesium chloride in physiological saline (0.9% sodium chloride). It is well known of course that either of these two salts when sufficiently concentrated (about 0.25%) will inhibit hemolysis. There is also reference (1, p.920) to the fact that either of these salts in very low concentration will assist hemolysis. Neither of these observations explains the apparently positive results which were obtained as above, *i.e.*, complete hemolysis in the absence of tubercle bacillus antigen, and no hemolysis when the antigen was present.

Characteristics of the Non-specific Reaction

1. *Both calcium and magnesium are required to produce non-specific reaction.* Used separately, appropriate concentrations (0.012%) of calcium chloride or of magnesium chloride produce weak non-specific reactions as indicated above, but it is only when both are present that the reaction is strong. The required ratio between the amounts of calcium chloride and of magnesium chloride is not critical; calcium chloride to magnesium chloride ratios between 4 to 1 and 1 to 2 are satisfactory. However, when the calcium chloride to magnesium chloride ratio is increased to 9 to 1, strong fixation of complement does not occur. In the following experiments we have used three parts (by weight of the anhydrous salts) of calcium chloride to one part of magnesium chloride, since this approximates the molecular calcium to magnesium ratio which is present both in the tap water originally used, and also in blood serum.

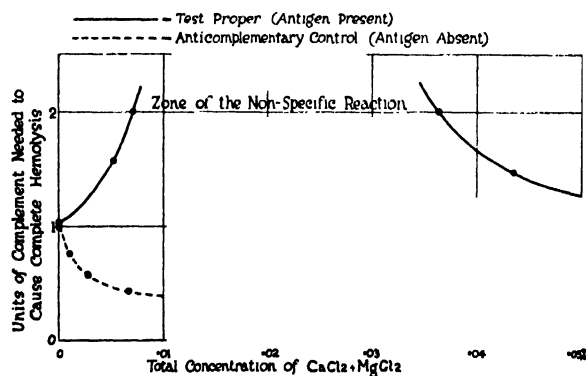


FIG. 1. Zone phenomenon produced by increasing amounts of calcium chloride and magnesium chloride.

2. *The Zone Phenomenon.* It is over only a very limited range of concentration of calcium chloride plus magnesium chloride that this non-specific reaction occurs, *i.e.*, when the total amount of calcium chloride plus magnesium chloride present is from 0.007% to 0.036%. This range varies somewhat with the antigenic value of the antigen employed, but the concentration of

*The tap water supplied to part of the city of London, Ontario, and to this Institution comes from springs. Its analysis, as furnished by the Public Utilities Commission, is as follows:—total solids, 287; calcium carbonate, 185; magnesium carbonate, 38; magnesium sulphate, 35.7; magnesium chloride, 1.0; silicates, 12.6; iron and aluminium oxides, 0.5; sodium chloride, 8.1; sodium nitrate, 12.1; ammonium chloride, 0.4. The figures given are in parts per million. It is of interest that with some antigens, *e.g.*, one made by boiling ground tubercle bacilli in water, this tap water gives much stronger non-specific reactions than does any solution containing only calcium chloride and magnesium chloride. Trial has shown that the bicarbonates and the soluble silicates of the water are responsible for increasing the non-specific reaction caused by the calcium chloride and magnesium chloride.

calcium chloride plus magnesium chloride which gives the strongest reactions remains constant at approximately 0.012%.

Fig. 1 shows the nature of this zone phenomenon of the non-specific T.C.F. reaction; it indicates the amount of complement which is required to cause complete hemolysis when varying concentrations of calcium chloride plus magnesium chloride are used in the presence and absence of antigen respectively. In the absence of antigen as little as one-third of a unit of complement may cause complete hemolysis when the concentration of calcium chloride plus magnesium chloride is 0.02%. With the presence of antigen in this same concentration of calcium chloride plus magnesium chloride, even four units of complement fail to cause hemolysis; in other words the results are those of a strongly positive test.

If the concentration of calcium chloride plus magnesium chloride exceeds 0.036% the test gives the correct negative result, *i.e.*, complete hemolysis both in the test proper and in the anticomplementary control. This persists until the concentration of calcium chloride plus magnesium chloride approaches 0.25%, when hemolysis is inhibited, regardless of the presence or absence of antigen, *i.e.*, the sample becomes anticomplementary. Over the range of concentration which gives this non-specific reaction there is present from 0.43 to 2.2 moles of calcium plus magnesium for each 100 moles of sodium. This is very significant, since solutions with the ratio of one or two moles of calcium for every 100 moles of sodium have distinctive physiological properties. Such solutions are referred to as "balanced physiological salines", since the destructive effect which either sodium chloride or calcium chloride alone exerts upon cell life is balanced and eliminated when the two salts are present in this ratio. The membranes of living cells, largely of lipoid nature, become more permeable to water when in a plain sodium chloride solution, while calcium salts have the reverse effect and greatly reduce the permeability of the cell wall. These opposite effects produced by calcium and sodium ions, briefly designated as "ion antagonism", can be demonstrated very clearly at oil-water interfaces, and are interpreted by Clowes (3) to result from the fact that the fatty acids of the oil form sodium and calcium salts which have opposite physical properties. The sodium salts are soluble in water and insoluble in oil, while the calcium salts are soluble in oil but insoluble in water. In the wall of the living cell other factors than these are also involved, but it is clear from the work of Loeb (7, p. 317), Osterhout (12, 13) and others that only when calcium and sodium salts are present in the ratio indicated above does the cell wall have that measure of permeability which is most favorable to cell life. The work of Clowes will be referred to later in an attempt to explain why the non-specific T.C.F. reaction occurs only in the presence of balanced saline.

The technique used for the T.C.F. test in these experiments is given in Table I. The volumes given are cubic centimetres.

After incubation for $1\frac{1}{2}$ hr. at 38° C. in the water bath, there is added to each tube:—amboceptor (2 units), 0.1 cc.; red cells (3% suspension), 0.2 cc. The samples are then returned to the water bath for 30 min. before reading.

TABLE I
TECHNIQUE USED FOR THE TUBERCULO-COMPLEMENT-FIXATION REACTIONS

	True positive test	Non-specific or false positive test	Negative test	Anticomplementary control on the non-specific test
Immune serum, suitably diluted	0.1	—	—	—
Complement (2 units)	0.2	0.2	0.2	0.2
Antigen (1 in 10)	0.1	0.1	0.1	—
0.09% CaCl ₂ in saline	—	0.1	—	0.1
0.03% MgCl ₂ in saline	—	0.1	—	0.1
Saline	0.6	0.5	0.7	0.6

Hemolysis is inhibited in both the true positive and the non-specific reactions.* Complete hemolysis occurs in the negative tests and in the anti-complementary controls. The solutions of calcium chloride and magnesium chloride indicated above are made by diluting isotonic (1.0%) solutions of these salts with plain saline to give the desired concentration. The term "non-specific reaction" is used in this paper to indicate this false positive reaction due to the presence of appropriate amounts of calcium and magnesium salts. The normal complement fixation reaction caused by immune serum is referred to as the "true positive reaction".

By the total concentration of calcium chloride plus magnesium chloride in a sample we refer to the concentration of these salts which is present during the preliminary 1½-hr. incubation of the antigen and complement, before the cells and amboceptor are added. The plain saline used in this work is 0.9% sodium chloride, while the term "balanced saline" as used here refers specifically to a solution containing 0.888% sodium chloride, 0.009% calcium chloride and 0.003% magnesium chloride, as it is at this concentration that the most pronounced non-specific reactions occur.

3. Incubation Period Required. In the true tuberculo-complement-fixation test a preliminary incubation of the antibody, antigen and complement for 60 to 90 min. at 38° C. is required for sensitization of the antigen and fixation of complement. Likewise in this non-specific reaction, this same period of incubation at 38° C. of complement, antigen and balanced saline is required. If this preliminary incubation is omitted, the calcium chloride plus magnesium chloride accelerate the hemolysis of the red cells, regardless of the presence or absence of antigen, so that the results are those of a negative test. Incubation for one and two hours at 20° C., or overnight incubation in the ice box, does not result in this non-specific fixation of complement.

Further information as to the nature of the non-specific reaction is obtained if only two of the three prime ingredients (complement, antigen, and calcium chloride plus magnesium chloride) are mixed and incubated for 1 or 1½ hr. at 38° C., the third ingredient being incubated alone, and then added to the other

*If the samples are centrifuged on completion of the test to remove the red cells, it can often be observed that inhibition of hemolysis is only 90% complete in the non-specific reaction.

two when the cells and hemolytic amboceptor are added. Using this procedure it is only when antigen and complement are mixed and incubated together that the non-specific reaction is obtained—the calcium chloride plus magnesium chloride being added along with the cells and amboceptor. When complement and balanced saline are mixed and incubated, or antigen and balanced saline are mixed and incubated, there is no inhibition of hemolysis on adding the third ingredient and the hemolytic mixture.

These results indicate an interesting point which was confirmed later by cataphoresis experiments, namely, that although no appreciable amount of complement is “fixed” when incubated with tubercle bacillus antigen in plain saline, nevertheless, union takes place between the antigen and some part of the normal guinea pig serum used for complement. This serum component, presumably protein, is irreversibly adsorbed on the antigen as will be shown later, and to a certain extent this corresponds to the sensitization of the antigen by immune tuberculosis serum. But while the truly sensitized antigen fixes complement in plain sodium chloride solution, the antigen normal serum complex fixes complement only in the presence of a balanced saline, *i.e.*, saline containing a certain concentration of calcium chloride plus magnesium chloride.

4. Complement Fixation in Undiluted Serum. Since undiluted blood serum is itself a balanced solution containing sodium, calcium and magnesium in approximately the concentrations used in this experiment, we would expect antigen to fix or inactivate complement when incubated with undiluted guinea pig serum. This was found to occur, the complement being completely inactivated.

However, exactly the same results were obtained when the calcium was first removed from the undiluted guinea pig serum by means of a slight excess of oxalate, so that this fixation of complement has no direct relation to the problem under discussion. Control tubes containing no antigen showed that the complement was not damaged nor rendered anticomplementary by the oxalate employed.

Accordingly, if it be found that the tubercle bacillus, or the products which it secretes in the tuberculous animal, inactivate complement *in vivo*, this cannot be attributed to the balanced proportions of calcium, magnesium and sodium ions in the body fluids.

5. Inhibition of the Non-specific Reaction by Citrate. Many biological and physical effects due to calcium ions may be eliminated by the use of an appro-

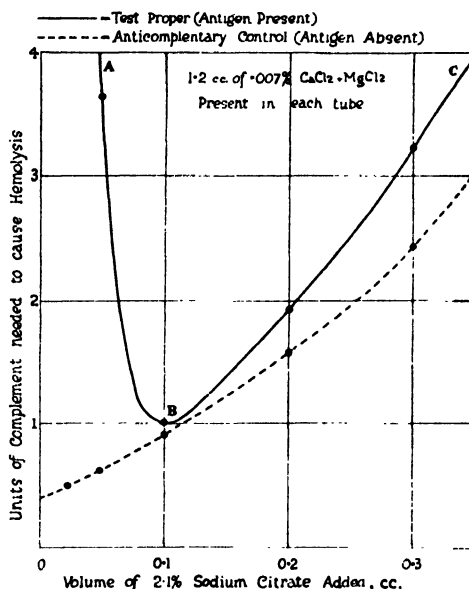


FIG. 2. Elimination of the false positive reaction by sodium citrate.

priate amount of sodium citrate. According to Marriott (9) citrate represses the ionization of calcium salts. Clowes (3) has shown that the same ratio between calcium chloride and sodium citrate is required to produce:— (a) zero effect upon oil-water interfacial tension; (b) minimum toxic effect when injected into mice; (c) inhibition of the clotting of blood; (d) elimination of the anti-hemolytic effect which is caused by either $M/10$ calcium chloride or $M/15$ sodium citrate when used separately. Trial showed that the non-specific T.C.F. reaction due to calcium chloride plus magnesium chloride could also be eliminated by the use of citrate, the result being shown in Fig. 2. The isotonic sodium citrate (2.1%)* was adjusted to pH 7.4 by adding a small amount of hydrochloric acid. Increasing amounts of this citrate were added to tubes along with the balanced saline, complement and antigen required to produce the false positive reaction. Anticomplementary controls containing no antigen were also prepared.

At the left of Fig. 2 at *A* is indicated the point where the calcium chloride plus magnesium chloride caused the typical non-specific reaction. With increasing amounts of citrate the non-specific reaction was eliminated as shown at *B*, and the sample then gave the correct negative result. Use of larger amounts of citrate caused an anticomplementary effect, shown at *C*, due to excess of citrate. Although the presence of suitable amounts of citrate prevented this non-specific reaction from taking place, the addition of citrate *after* the non-specific reaction had occurred (*i.e.*, after 1 or $1\frac{1}{2}$ hr. incubation of balanced saline, complement and antigen) produced no effect. In other words the complement was irreversibly fixed by the antigen. Similarly, if after the non-specific reaction the calcium was removed by oxalate precipitation, the antigen did not give up the adsorbed complement in an active state, as was indicated by lack of hemolysis. The writer has not in this work encountered any condition whereby complement which has been "fixed" by tubercle bacillus antigen can be "desorbed" and recovered in its active state.

Clowes (3) used sufficient citrate to convert an isotonic solution of calcium chloride into (so far as calcium ions were concerned) a physiologically balanced solution. In his non-specific reactions the writer starts with a balanced saline, and in the above experiment added citrate until the balanced saline reacted like plain sodium chloride. The appropriate calcium to citrate ratio in this experiment is accordingly entirely different from that found in the work of Clowes.

6. *Concerning the Antigen.* Only those tubercle bacillus antigens which are partly or entirely lipoidal in nature give this non-specific reaction. By extracting† ground bacilli for three successive periods of $1\frac{1}{2}$ hr. each with

*There is some divergence of opinion upon isotonic strengths of solutions. From our present point of view, a solution is regarded as isotonic when just sufficiently strong that it prevents hemolysis of sheep red cells during 30 min. incubation at 38°C . Arbitrary rules such as those which take $M/10$ or $M/8$ concentrations of divalent salts, or $M/15$ solutions of trivalent salts, as being isotonic, have caused much confusion in serology. For example, sodium sulphate is reported to have an anticomplementary effect (1, p. 920), which is true for $M/8$ or $M/10$ concentrations. But a 1.1% solution of sodium sulphate (0.0775 M) causes no anticomplementary effect even when this solution forms 50% of the total volume in the T.C.F. test, nor does this alter the titre of the sample.

†This was done in a Soxhlet type of extraction apparatus which operated under reduced air pressure to lower the boiling points of the solvents. A description of this apparatus will be published in the *Journal of Biological Chemistry* for December, 1932.

acetone, chloroform and methyl alcohol at 35° C., a "fat-free" residue antigen was obtained which was efficient in the true T.C.F. reaction, but failed entirely to give these non-specific reactions. If the acetone or methyl alcohol extracts were added to the "fat-free" antigen, it resumed its ability to give non-specific reactions. The same result was obtained, although to a less pronounced degree, if small amounts of olive oil or heavy petroleum were emulsified in the "fat-free" antigen.

The antigen used most frequently in this work is similar to the whole bacillus antigen of Petroff (15); the writer used twice the amount of ground tubercle bacilli that Petroff's directions call for, and used the antigen in a dilution of 1 in 10 instead of 1 in 30*. The diluted antigen was centrifuged for 15 min. at 1,800 r.p.m., and only the supernatant liquid used. The anticomplementary effect of the amount of this antigen used in these experiments was not more than 0.1 unit of complement, and was usually appreciably less than this.

The method used in preparing the Petroff type antigen is as follows: the ground bacilli are extracted with toluene, and from the mixture of bacillary residue and extract the toluene is allowed to evaporate, thus forming a coating of lipoidal material on the bacillary fragments. It is probably on account of the large lipoidal surface exposed that the Petroff type antigen gave this non-specific reaction much more distinctly than did any other type of tubercle-lipoid antigen.

By using smaller amounts of antigen, this non-specific reaction could be weakened or eliminated. Usually 0.1 cc. of the centrifuged 1 in 10 antigen was required to assure strong non-specific reactions, although as little as 0.0025 cc. of this same dilution of antigen fixed two units of complement when sensitized with a very strongly immune tuberculosis serum. It was observed that after aging the Petroff type antigen for two months its antigenic power in the true T.C.F. reaction was essentially unchanged, while its ability to cause the non-specific reaction had largely disappeared.

A sample of Wassermann antigen, which was tried in a wide range of concentration, failed to give any non-specific complement fixation in the presence of balanced saline. A tentative explanation for this will be indicated in a later paper.

7. Inhibition of Hemolysis by Excess of Complement. In the presence of balanced saline and antigen, the addition of increasing amounts of complement at times inhibits hemolysis. When one unit of complement is present it produces hemolysis, whereas if two, three or four units of complement are present no hemolysis occurs. As previously noted, all these samples are incubated 1½ hr. at 38° C. before adding the sensitized red cells. This phenomenon has occurred frequently in the course of this work, but not with perfect regularity, nor can the factors necessary to produce this result be stated with certainty. Efforts to produce this same effect by keeping the

**The writer has always found that a considerable portion of the ground bacilli was too coarse to stay in colloidal suspension; a further portion of the coarser particles is removed by centrifugation and discarded. Hence the antigen employed is probably not more concentrated than that used by Petroff, who appears to have dispersed almost all of the tubercle bacilli into a stable colloidal state.*

volume of complement constant and adding increasing amounts of inert serum (human serum albumin or normal rabbit serum) were unsuccessful. The usual effect of inert serum was to inhibit, partly or completely, the non-specific reactions described in this paper. However, this type of prezone phenomenon occurred more consistently and clearly when 0.2 cc. of a 10% fresh egg-white

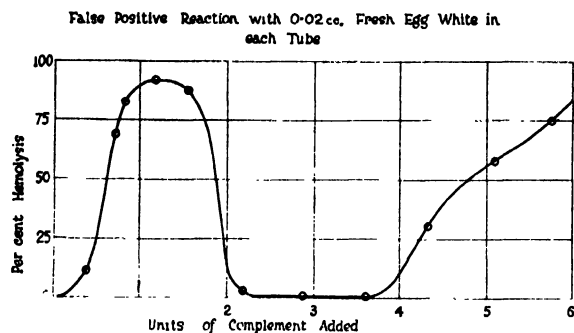


FIG. 3. *Inhibition of hemolysis caused by excess of complement.*

thousands of routine T.C.F. tests on the sera of tuberculous patients in the Queen Alexandra Sanatorium, no such effect was ever observed.

8. *Calcium Requirements of the True Complement Fixation Reaction.* It is important to know whether the presence of calcium salts is essential to the true complement fixation reaction. To examine this, the small amount of calcium present in all the normal reagents for the T.C.F. test, *i.e.*, antigen, amboceptor and complement, was removed by adding sufficient potassium oxalate to completely precipitate the calcium. After two hours the calcium oxalate precipitates were removed by centrifugation. The red cells were allowed to stand for two hours in saline containing 0.1% potassium oxalate, and were then well washed in plain saline. On titrating these reagents it was found that they all reacted normally, and showed no appreciable change in strength. A sample of known positive serum, from which the calcium had been similarly removed, was then tested with these calcium-free reagents; it gave the normal positive reaction, without anticomplementary effect. Accordingly, the presence of small amounts of calcium in the reagents or sample is not essential for the true T.C.F. reaction.

9. *Effect of Balanced Saline on the True Complement Fixation Reaction.* In the presence of balanced saline and a small amount of antigen (*e.g.*, only one-tenth of the antigen needed to give a false positive reaction), the normal T.C.F. test upon immune serum may be carried out. The presence of the calcium chloride plus magnesium chloride does not increase the amount of complement fixed by the sensitized antigen, but apparently has the reverse effect. When successive dilutions of antigen which had been sensitized by immune serum were incubated for one hour with two units of complement, both in plain saline and in balanced saline, it was found that with the balanced saline twice as much sensitized antigen was needed to inhibit hemolysis as was needed when

solution was present in each tube of the non-specific T.C.F. reaction. As shown in Fig. 3, one unit of complement caused hemolysis, two, three or four units of complement inhibited hemolysis, while still larger amounts of complement again caused hemolysis. These inhibitions by excess of complement have not been investigated further by the writer, who has no satisfactory explanation for them. In the course of some

plain saline was used. It is possible that these results do not indicate that balanced saline tends to inhibit the fixation of complement by the sensitized antigen. As indicated earlier, complement is almost three times more effective in causing hemolysis when in balanced saline than when it is in plain saline, and this may be the cause of the greater hemolysis observed in the balanced saline in the above experiment.

Part II. Theoretical Considerations

The observed facts in connection with this non-specific reaction have been described in the foregoing section. Table II gives the concentrations of metallic ions in Ringer's solution (17), the balanced solutions used to produce the non-specific complement fixation reaction, and human blood serum (6, p. 357). Ringer's solution contains no magnesium salts, but has a calcium concentration

TABLE II

CONCENTRATIONS OF METALLIC IONS IN RINGER'S SOLUTION, THE BALANCED SOLUTIONS USED TO PRODUCE THE NON-SPECIFIC COMPLEMENT FIXATION REACTION, AND HUMAN BLOOD SERUM

	Na	K	Ca	Mg
	Molar concentration			
Ringer's solution	.1300	.0002	.0011	—
Balanced saline used to produce the non-specific T.C.F. reaction	Lowest Ca and Mg concentration			
	.152	—	.0005	.0002
	Optimal Ca and Mg concentration			
	.152	—	.0008	.0003
	Highest Ca and Mg concentration			
	.152	—	.0025	.0010
Human blood serum	.144	.005	.0025	.0010

exactly equal to the total calcium plus magnesium concentration which gave the strongest non-specific T.C.F. reaction. The highest concentrations of calcium and magnesium which gave the non-specific reaction are the same as the calcium and magnesium concentrations in blood serum.

There remains to be given a tentative answer to the questions:— why does the non-specific reaction occur only over this limited range of calcium and magnesium concentration?; and, why is this also the range of concentration which biologists have repeatedly found to be essential to cell life?

Since the non-specific T.C.F. reaction occurs only with lipoid antigens, and the membranes of living cells are also largely lipoidal in nature, the writer quotes from the work of Clowes (3) who studied the effects of ion antagonism at oil-water interfaces. It is to be understood, of course, that when a full knowledge of the nature of ion antagonism is available it will include phenomena which are not related to colloids or lipoids, as shown by the work of Simms (16), Pascoe (14) and others.

That particular ratio of sodium to calcium and magnesium which is optimal for producing the non-specific T.C.F. reaction is also approximately that ratio which is essential to many forms of cell life. Again, a mixture of these salts

in the above ratio has no effect upon the stability and surface tension of oil-water interfaces, although when used separately each of these salts disturbs conditions in an oil-water system. It appears probable that these three phenomena, occurring as they do in each case at an essentially lipoid-water interface and at the same ratio of sodium to calcium and magnesium, are manifestations of one underlying cause.

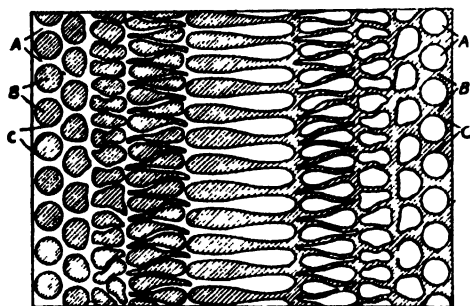


FIG. 4. Diagrammatic representation of the state of unstable equilibrium when neither oil-in-water nor water-in-oil type of emulsion predominates. A = water; B = oil; C = adsorbed film.

It has been shown by Clowes that while sodium oleate stabilizes an oil-in-water emulsion and calcium oleate stabilizes a water-in-oil emulsion, a mixture of these two salts in suitable proportion gives rise to an unstable system in which the two phases separate rapidly, as no stable emulsion is formed. These relations are shown in Fig. 4, which is reproduced from the article by Clowes. Upon these oil-water systems, sodium chloride and calcium chloride exert antagonistic effects, approximately 100 moles of sodium chloride tending to stabilize an oil-in-water emulsion to the same degree that one mole of calcium

chloride tends to invert the phases and stabilize a water-in-oil emulsion. Provided that sodium chloride and calcium chloride are added in this ratio of 100 to 1, no effect is produced upon the oil-water system. Referring to this work by Clowes and its bearing upon changes in the permeability of the cell wall produced by sodium or calcium ions, Gortner (4, p. 273) states, "Clowes interprets these results as changes in protoplasmic emulsions, due to the effect of the specific ions, regarding the plasma membrane as an emulsion fairly closely balanced between oil-in-water and water-in-oil types, the NaCl causing a more complete peptization of the cell membrane and forming a more perfect oil-in-water type, the CaCl_2 causing inversion to a water-in-oil type. Eventually (excess of) either CaCl_2 or NaCl breaks the emulsion causing a separation of the two phases with the ensuing death of the organism."

In the intricate lipid-protein complex which constitutes the living cell membrane, the state designated above as "an emulsion fairly closely balanced between oil-in-water and water-in-oil types" appears to be the counterpart of that state, in the simple oil-water system described by Clowes, in which neither an oil-in-water nor a water-in-oil type of emulsion predominates. However, in the last sentence of the above quotation Gortner completely misinterprets the experimental evidence described by Clowes. While it is true that under appropriate conditions solutions of either sodium chloride or calcium chloride will crack an oil-water emulsion, such solutions are outside the conditions and the range of concentrations which are under discussion. Slight changes of either sodium or calcium concentration from the critical 100 to 1 ratio do *not*

crack the emulsion, but *stabilize* the emulsion. Life is essentially a condition of unstable equilibrium, an equilibrium which is finely adjusted and, to a limited extent, under the control of the living organism. Any treatment which results in the formation of an equilibrium which is stable, and hence beyond the ability of the organism to control and adjust, would cause death of the organism.

On these grounds the critical ratio of sodium to calcium which obtains in balanced physiological solutions appears to be essential to cell life because it maintains the cell membrane in a state of unstable equilibrium. Solutions which do not contain balanced proportions of the antagonistic sodium and calcium ions convert the cell membrane into a stable phase, the properties which the organism cannot manipulate to meet its requirements, and this leads to the death of the organism.

Relating this to the non-specific complement fixation reaction, two points must be kept in mind:

1. That in all true complement fixation reactions, the sensitized antigen particles acquire a tendency to agglutinate or cohere at the same time that they acquire the ability to fix complement.

2. As will be described in detail in a second paper, in the non-specific T.C.F. reaction (*i.e.*, in the presence of balanced saline) the antigen likewise acquires the power to agglutinate and to fix complement.

The experiments by Northrop and De Kruif (11) and later by Mudd (10) have shown the relation of the "cohesive force" which leads to agglutination to the degree of sensitization of the antigen or bacteria. So far as the writer can find, the nature of this "cohesive force" has never been explained, but would suggest that the presence of the appropriate ratio of sodium to calcium and magnesium (*i.e.*, balanced saline) in the non-specific reaction may produce a condition of unstable equilibrium, similar to that described above for the living cell membrane, at the lipoid-protein surfaces of the antigen. It has also been shown by experiment that it simultaneously confers on the antigen cohesive and complement fixing properties. Hence the writer suggests that the "cohesive force" which leads to agglutination of antigen particles in the non-specific reaction is the outcome of a state of unstable equilibrium being produced at the surface of the antigen. An oil-water emulsion, under such conditions as lead to an unstable equilibrium, separates quickly, the small drops uniting into larger aggregates until the oil and water phases have completely separated, and analogous to this there is in the non-specific T.C.F. reaction the agglutination of antigen particles, with concomitant fixation of complement.

Hence the writer tentatively suggests that the non-specific complement fixation reaction occurs at a definite sodium to calcium (plus magnesium) ratio because this ratio is such as to produce at the surface of the antigen particles that state of unstable equilibrium which leads to agglutination, and hence complement fixation.

The writer does not know why the presence of both magnesium and calcium gives stronger non-specific reactions than does either of these alone. A parallel observation was made by Loeb (7, p. 317) that both calcium and magnesium salts are essential to the well-being of certain marine organisms. Of course,

the surface of the antigen is not a simple oil-water interface such as Clowes studied, but is probably a lipoid-protein adsorption complex, and the effect of the magnesium may be due to its peculiar abilities to react with amino acids and hence with proteins (Zörkendörfer, 18).

Referring to the cohesiveness produced in antigens by immune serum, the writer has no evidence to suggest whether this is due to some factor entirely distinct from the unstable surface equilibrium indicated in the non-specific reaction. It would be of great interest to measure the cohesive force between those unicellular organisms which are sensitive to deviations from the appropriate sodium to calcium ratio, both when these organisms are suspended in plain saline and when in balanced physiological saline. The view which is presented here would be in part confirmed if, as would be expected from the foregoing, cohesiveness is greater in the balanced saline. Mudd's resuspension method (10) would be better adapted to these measurements than Northrop and De Kruif's (11) torsion balance method, since in the latter it is necessary to dry the organisms and heat them to 60° C.

It was pointed out earlier in this paper that in the presence of balanced saline the hemolytic power of complement is almost three times greater than when the complement is used in plain sodium chloride solution. Similarly, Andreesco (2) has shown that small amounts of sodium bicarbonate increase the hemolytic efficiency of complement. This suggests that the blood, which contains both sodium bicarbonate and balanced proportions of sodium to calcium and magnesium, maintains suitable conditions for assisting native complement to exert its greatest efficiency, and that some or all of the tests for measuring *in vitro* the bacteriolytic power of immune sera and complement should be carried out in Ringer's solution.

In conclusion, it should be pointed out that this article does not criticize the validity of the routine tuberculo-complement-fixation test, as the conditions normally used in this test do not lead to this non-specific reaction.

Summary

1. A non-specific or falsely positive tuberculo-complement-fixation reaction is described.
2. The use of lipoidal antigens, and the presence of a balanced physiological saline, similar to Ringer's or Tyrode's solution, is needed to produce these non-specific reactions.
3. A tentative hypothesis, based upon physico-chemical effects at oil-water interfaces, is advanced to explain why the particular sodium to calcium ratio in balanced salines is essential both to the well-being of cell life and also to the production of the non-specific complement fixation reaction herein described.

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ON THE MECHANISM OF COMPLEMENT FIXATION¹BY A. HAMBLETON²

Abstract

As previously described, a falsely positive or non-specific complement fixation reaction can be produced with certain tuberculo-antigens; the mechanism of the reaction is described in the present article. Examination of the antigen in the non-specific reaction shows that it can assume the same complement fixing power, cohesiveness and oil-wettability as antigen sensitized by immune serum. This indicates that fixation of complement is not a specific immune reaction, but only a secondary effect due to the surface properties of suitably modified antigens.

In those immune reactions which produce fixation of complement, it is known that both agglutination of the antigen and fixation of complement are secondary stages in the reaction. The first stage involved is the sensitization of the antigen by homologous immune serum, a reaction which is usually highly selective. To explain this highly selective or specific action, it appears necessary to assume some specific chemical affinity between the antigen and its homologous immune substance. But with reference to the subsequent fixation of complement, it is not clear whether this also is a specific immune reaction, or whether it is a purely physical adsorption. Eagle (2) states, "As yet it is unknown whether this adsorption (of complement by sensitized antigens) is determined by the physical state of the (agglutinated antigen) precipitate, and thus differs only quantitatively from that by kaolin, charcoal, normal bacteria, heat denatured proteins, etc.; or whether the comparatively enormous avidity of the sensitized antigen for complement is due to a specific chemical affinity".

In this paper will be presented data which, within the accuracy of available experimental methods, give an answer to this statement by Eagle.

The basis for these data has been indicated in another paper (4) describing a non-specific complement fixation reaction, where it was shown that in the presence of a balanced physiological saline, akin to Ringer's or Tyrode's solution, the antigen used in the tuberculo-complement-fixation test acquires the power to fix complement without the presence of specific antibody. In other words, the antigen in this non-specific reaction acts as does an antigen sensitized by homologous immune serum. With one and the same antigen it is thus possible to produce two similar complement fixation reactions, one by means of immune serum and the other by non-specific means. This makes possible a comparison of the surface properties of the antigens from these two reactions, under such conditions that they exhibit equal complement binding power. There is ground for assuming that an antigen sensitized by immune serum has a specific chemical affinity for complement only when this sensitized antigen possesses a greater complement fixing power than can be accounted

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for by its other physical properties. In view of the simple and non-specific reagents which are used to produce the non-specific complement fixation reaction, no specific chemical affinity for complement can be ascribed to the antigen in this test. Hence if this antigen, treated by entirely non-specific reagents, assumes the same physical properties and the same complement fixing power as the truly sensitized antigen, the latter clearly does not exert any specific chemical affinity for complement.

The data presented in this paper constitute a comparison of the physical properties of the specific and non-specific antigens under such conditions that they exert equal complement binding power. Within the accuracy limits of the tests which have been employed, the two antigens are shown to have in many respects identical physical properties. To make these results clearer, the nature of the non-specific reaction will first be briefly indicated.

Mechanism of the Non-specific Reaction

To produce the non-specific reaction, the antigen and diluted complement must be incubated together for at least one hour at 38°C. If these ingredients are incubated in a plain 0.9% sodium chloride solution, complement fixation does not occur, but the antigen is so modified that on adding the trace of calcium chloride and magnesium chloride which is needed to convert the plain saline into balanced saline, complement is fixed almost instantly, and the antigen shows a marked tendency to agglutinate. Further details on this point are covered in a preceding paper (4).

TABLE I

	Antigen control	Negative reaction	Non-specific or false positive reaction	True positive reaction
Antigen, 1 in 10, cc.	3	3	3	3
Complement, 1 in 10, (2 units), cc.	—	6	6	—
Immune guinea pig serum, 1 in 10, cc.	—	—	—	6
CaCl ₂ (0.09% in 0.9% NaCl), cc.	—	—	3	—
MgCl ₂ (0.03% in 0.9% NaCl), cc.	—	—	3	—
Plain saline (0.9% NaCl), cc.	27	21	15	21
Total volume, cc.	30	30	30	30
Isoelectric point of the antigen, washed four times in plain saline, pH	3.2	3.7	3.7	4.9

To examine changes in the surface properties of the antigen, samples were made up according to Table I, so as to produce negative, non-specific, and true positive complement fixation reactions, and along with these was a control of antigen suspended in plain saline. After 1½ hr. incubation at 38° C. the samples were kept in the ice box overnight and then the antigen in each tube was thrown down by centrifugation. These antigen sediments were washed four times in plain saline, and then resuspended in portions of 0.045% sodium chloride which had been adjusted by addition of hydrochloric acid to give

various pH values. Cataphoresis was carried out in an improved form of the Northrop cataphoresis cell, observing the precautions outlined by Kahn and Schwarzkopf (5).

The washed, untreated antigen in the control was found to have its isoelectric point at pH 3.2. Antigens from both the negative and the non-specific reactions showed the same isoelectric point at pH 3.7, while the antigen from the true positive reaction was isoelectric at pH 4.9.

This result indicates:—

1. The surface properties of the antigen are modified even in the negative reaction, *i.e.*, where antigen and complement are incubated together in plain saline, since the isoelectric point of the antigen is changed from pH 3.2 to pH 3.7. Presumably this change in properties is due to an irreversible adsorption of serum protein upon the antigen, and is similar to that described by Mudd (7) in his extensive work upon tubercle bacilli, wherein he states "The bacterial surfaces are modified by treatment with fresh normal sera in a manner quantitatively less, but qualitatively indistinguishable by the methods used, at least, from the effects of immune sera".

2. That if the presence of traces of calcium and magnesium in the non-specific reaction causes a further change in surface properties, as seems probable since complement is fixed, no evidence of this change is shown by cataphoresis measurements after the antigen has been washed in plain saline.

Agglutination tests were employed to examine whether the surface properties of the antigen from the non-specific reaction are reversible, *i.e.*, whether the special characters possessed by the antigen when suspended in balanced saline are eliminated if the antigen is suspended in plain saline. As the direct agglutination test is never strong with these antigens, and results are often further obscured by a prezone effect, we have used the modified form of test designated by Mudd (6) as the resuspension test. In this test the samples are centrifuged at high speed to pack together the antigen particles. After removing the supernatant fluid, a small volume of saline is added to the antigen sediment, and upon shaking the tubes uniformly in a rack, the antigen is resuspended in the saline. The coarseness or size of flocculi in this suspension gives a roughly quantitative measure of the cohesiveness of the antigen. The agglutinating or cohesive tendency of the antigen particles is of special significance in this work since the correlation between the phenomena of agglutination and complement fixation is well established.

The tests were carried out upon the antigen sediment from a non-specific reaction which showed marked cohesiveness in comparison with the antigen from a negative T.C.F. reaction. This cohesive antigen from the non-specific reaction was divided in two portions; one of these was well washed in balanced saline, and this portion retained its cohesiveness; the other portion of antigen was washed in plain saline, and was then found to have very little cohesiveness when, after these washings, the two antigens were compared by the resuspension test.

This proves that the cohesiveness of the antigen-protein complex which is induced by the traces of calcium and magnesium present in balanced saline

is a reversible property which is lost when the antigen is suspended in plain saline. Nevertheless, as pointed out in another paper (4), fixation of complement cannot be regarded as a reversible process, for neither in the specific nor the non-specific reactions has it been possible to elutriate in an active form the complement which has been fixed by the antigen.

Accordingly, the factors which produce the non-specific reaction are as follows:

1. The surface properties of the antigen are irreversibly modified* by incubation in the presence of dilute complement.

2. These surface properties are further modified by the traces of calcium and magnesium present in balanced saline, so that the antigen

- (a) fixes complement,

- (b) agglutinates or coheres markedly.

Since in true complement fixation reactions induced by immune serum the fixation of complement and the cohesiveness of the antigen are also associated phenomena, it seemed desirable to quantitatively compare these two factors, complement fixation and agglutination, as they occur in the true and in the non-specific T.C.F.† reactions. Later two other properties of these antigens were also investigated, *i.e.*, the partition of the antigen between oil and water phases, and the electrical potential of the antigen particles as measured by cataphoresis.

Comparison of the Physical Properties of the Antigens in the True and the Non-specific T.C.F. Reactions

The non-specific reaction cannot easily be adjusted to give any desired degree of complement fixation, as is the case with the true complement fixation test. In the latter test, by varying the amount of immune serum, the intensity of the reaction can be varied at will. Accordingly the method employed was as follows:—

1. By preliminary titration of the non-specific reaction, the smallest quantity of antigen was found which would inhibit hemolysis in the presence of two units of complement.

2. An equal quantity of antigen was then employed, along with two units of complement, in the true complement fixation reaction, and the smallest dose of immune serum was determined which, under these conditions, would just inhibit hemolysis. For brevity this amount of immune serum will be referred to as a unit dose.

3. Seven samples were then made up as shown in Table II. For convenience, these samples are 30 times as large as those used in the preliminary tests, where the final volume was 1 cc. In five of these samples we have true positive reactions, using successively 4, 2, 1, 0.5 and 0.25 doses of immune serum

* In contrast to this it is interesting to note that Eagle (3) and others (13), have shown that in the negative Wassermann reaction only a loose reversible adsorption takes place between the antigen and the normal serum; after two or three washings with saline the adsorbed protein is removed, the antigen apparently resuming its original surface properties. This may explain why Wassermann antigen does not give non-specific fixation of complement in the presence of balanced saline.

† Tuberculo-complement-fixation.

per cc. Due to the antigen being present in large excess, these samples are then capable of fixing approximately proportionate amounts of complement, *i.e.*, 8, 4, 2, 1 and 0.5 units. The last two tubes, samples 6 and 7, are duplicates of the non-specific reaction. All samples were incubated $1\frac{1}{2}$ hr. at $38^{\circ}\text{C}.$, and then stood overnight in the ice box. The following tests were then applied to the antigens in these samples.

(a) *Agglutination Test.* Direct and ultramicroscopic examination of the samples gives little information. There is no macroscopic agglutination; slight agglutination is observed in all tubes by dark field illumination, but the difference in the degree of agglutination is not appreciable. Hence the value of the resuspension test to measure the cohesiveness of the antigen particles.

(b) *Resuspension Test.* The samples were centrifugated 30 min. at 3000 r.p.m. and the supernatant fluids, with the exception of 0.25 cc., were removed and discarded. Each tube thus contained the packed antigen sediment along with this 0.25 cc. of the corresponding supernatant liquid. The tubes were all placed in one rack which was gently shaken until the antigen was uniformly and finely suspended in that sample, No. 5, which received least immune serum. The contents of each tube was then poured into small watch glasses for examination.

TABLE II

Sample number	1	2	3	4	5	6	7
Antigen (dilution found by preliminary titration), cc.	3	3	3	3	3	3	3
Complement, 1 in 10, cc.	6	6	6	6	6	6	6
Immune sheep serum, (dilution found by preliminary titration), cc.	16	8	4	2	1	—	—
CaCl_2 (0.09% in 0.9% NaCl), cc.	—	—	—	—	—	3	3
MgCl_2 (0.03% in 0.9% NaCl), cc.	—	—	—	—	—	3	3
Plain saline (0.9% NaCl), cc.	5	13	17	19	20	15	15
Total volume	30	30	30	30	30	30	30

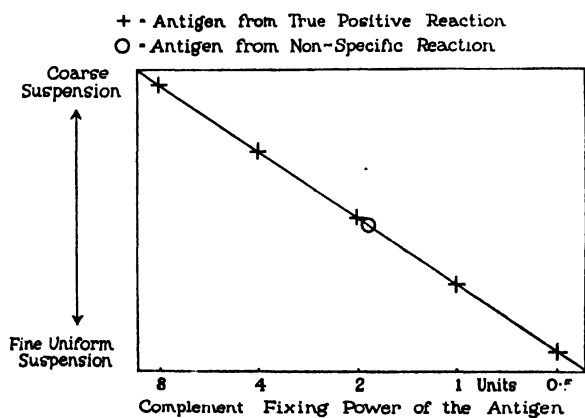


FIG. 1. Comparison of the cohesiveness of the antigens from the true positive and the non-specific reactions.

Sample No. 1, which received the largest amount of immune serum, consisted of large flakes of antigen suspended in a relatively clear liquid, and the samples Nos. 1 to 5 formed a series in which the fineness of the flakes of antigen increased until in sample No. 5 a fine uniform suspension of antigen was present. Samples Nos. 6 and 7, duplicates of antigen from the non-

specific reaction, were then compared with this series. In all cases samples Nos. 6 and 7 corresponded closely in degree of fineness with sample No. 3; if any slight distinction could be made between these three samples, Nos. 6 and 7 were a shade more finely divided, and hence may have exhibited slightly less cohesion than sample No. 3. This matching was always assigned to workers who had no knowledge of the nature of the samples, so as to eliminate prejudice on the part of the observer. By the preliminary titration samples Nos. 3, 6 and 7 had been so prepared that each fixed exactly the same amount of complement, *i.e.*, two units, and this test indicates that the antigens in these three samples also possess a similar degree of cohesiveness. This result is shown graphically in Fig. 1.

(c) *Oil-water Partition of the Antigen.* It was shown by Mudd (8, 9, 10) that when acid-fast bacteria are sensitized with immune serum, their tendency to pass from a water into an oil phase is reduced. This test has been made more adaptable to quantitative measurements by Reed and Rice (15); their method consists of shaking an aqueous suspension of the bacteria with oil, and separating the oil and water phases by centrifuging at low speed. Bacterial counts of the aqueous phase, before and after shaking with the oil, indicate the relative partition of the bacteria between the oil and water phases.

The antigen used in the T.C.F. reactions under discussion is essentially a stable suspension of finely ground tubercle bacilli (14), and trial showed that it was not possible, by the dark-field illumination employed, to count the number of antigen particles, since these vary greatly in size, some being barely visible, and probably many of them being completely invisible. Nor was it possible to estimate the amount of antigen in the water phase on the basis of turbidity, since the amount of antigen employed gives but slight turbidity to the samples, and a certain degree of turbidity is imparted to an aqueous phase upon vigorously shaking with oil.

Accordingly, the percentage partition of the antigen to the oil phase was estimated by titrating the amount of antigen in the aqueous phase before and after shaking with oil. The method used was as follows. Samples were made up as indicated in Table II. After the $1\frac{1}{2}$ -hr. incubation the samples were cooled, and a portion of each removed. To the remainder of each sample was added an equal volume of washed heavy petroleum oil, and these samples were then shaken for 30 min. in the mechanical shaker. After separation, the water phase was siphoned from below the oil. The antigenic content of each sample, before and after shaking with the oil, was found by incubating for one hour at 38°C. serially decreasing volumes of sample with 0.1 cc. of a strongly immune serum and three units of complement; the hemolytic mixture was then added and the samples returned to the water bath for 30 min. Controls showed that the samples acquired no significant anticomplementary properties due to being shaken with the oil. The results are shown in Fig. 2. This reaction falls short of the desired degree of sensitivity, as in the true positive tests all samples which fix from 0.5 to 4 units of complement show the same degree of partition to the oil phase. It is clear, however, that this same degree of partition is also shown by the antigens from the non-specific

reaction, which fix two units of complement. Hence those antigens from the non-specific and the true positive reactions which have equal complement binding power also show similar degrees of partition between oil and water.

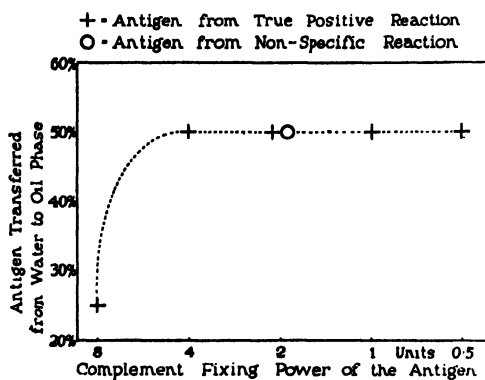


FIG. 2. Comparison of the oil-water partition of antigens from the true positive and the non-specific reactions.

The lack of sensitivity in this test is probably due to the inert serum present in the samples. To minimize this effect, in another experiment samples Nos. 1 to 5 were diluted with three volumes of plain saline, and samples Nos. 6 and 7 were diluted with three volumes of balanced saline before shaking with the oil. Results however were essentially unchanged. It is not feasible to wash the antigen and suspend it in saline before shaking with the oil, since the antigen under these conditions agglutinates into coarse flakes which accumulate

almost entirely at the oil-water interface.

(d) *Cataphoresis*. With the sensitization of acid-fast bacteria there occurs a reduction in the electrical potential of the organisms. Extensive quantitative data have been presented by Mudd and coworkers (11) on this point. It was desirable to know whether the antigen from the non-specific reaction had the same electrical potential as the antigen from a truly specific reaction, when the two antigens exhibited the same degree of complement binding power. Samples were made up similar to those indicated in Table II, except that a somewhat greater range of immune serum concentration, from 6 down to 0.188 doses, was used in the true positive samples. After incubation, the samples were kept overnight in the ice box and the antigen was thrown down by centrifugation. The antigen sediments from the true immune reactions were washed and resuspended in plain saline, while the antigens from the non-specific reaction were washed and resuspended in balanced saline, as it has been shown that the latter antigens retain their characteristic properties only as long as they are in contact with balanced saline. The final suspensions were maintained at the approximately uniform pH of 7.0 by the presence of 0.001 M phosphate buffer. The washing of this ground bacillus antigen is made difficult

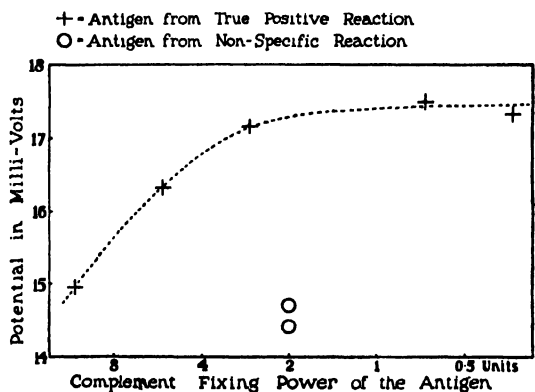


FIG. 3. Comparison of the electrical potential of the antigens from the true positive and the non-specific reactions.

by the cohesiveness of the particles, so that even prolonged grinding leaves much of the antigen in the form of a coarse suspension. After the second washing these coarser particles were removed by slow centrifugation, and cataphoresis was carried out upon the finer particles which remained in suspension. The electrical potential of untreated antigen particles is extremely uniform, and it is of interest that this potential is essentially the same in plain saline or in balanced saline, the observed difference of 0.5% being less than the limit of accuracy of the method employed. The treated and washed antigen particles do not show great uniformity of electrical potential. The results obtained are indicated in Fig. 3, where a curve has been drawn through the crosses which indicate the potentials on the samples which received serial doses of immune serum. The potentials upon the antigens from the duplicate non-specific reactions are seen to be remote from the corresponding potential upon the antigen from the true positive reaction. In this respect, then, the non-specific reaction does *not* closely simulate the true immune reaction.

Discussion

It would have been preferable to compare the surface properties of the specific and the non-specific antigens before they adsorbed complement. This could not be done in the non-specific reaction, since in the absence of complement the antigen exhibits none of those interesting surface properties which it assumes in the presence of complement and balanced saline. Accordingly the surface properties, both in the true and in the non-specific reactions, have been compared in samples containing two units of complement.

It was observed by Olitzki (12) that with certain bacterial types which agglutinate with homologous immune serum in the form of fine-grained precipitates, that the degree of agglutination was increased by the presence of small amounts of complement. Other bacterial types failed to show this effect. In this work with tuberculo-antigens the author has not observed the effect described by Olitzki, but rather the reverse effect, namely, that the presence of complement, or inert serum, reduces the amount of visible agglutination, and may give a prezone effect. This prezone effect in the direct agglutination reaction does not occur in the resuspension test. A detailed examination of the surface properties of sensitized antigens, before and after being exposed to complement, apparently has not been described in the literature. It is clear, however, that fixation of complement causes no marked effect on the cohesive power of the antigens in the true T.C.F. reactions which we have examined. Hence in this regard the surface properties of the antigen in the presence of two units of complement, as described in this article, are a fair index of the surface properties of the antigen which has not been exposed to complement.

The data presented have shown that by treatment with non-specific reagents, namely, balanced physiological saline and normal guinea pig serum, the antigen has acquired a set of physical properties which are very similar to those of an antigen sensitized by immune serum. So far as the limited accuracy of the methods available can indicate, those specific and non-specific

antigens which fix equal amounts of complement also possess the same degree of cohesiveness and oil-wettability, but do not possess the same electrical potential.

Since we cannot ascribe to the antigen in the non-specific reaction, on account of the simple and non-specific reagents employed, any "specific chemical affinity for complement", it is clear that there is little or no ground to claim a "specific chemical affinity for complement" for the antigen sensitized by immune serum, as the two types of antigen have similar physical properties and the same ability to fix complement. The one marked divergence between the two types of antigen is in their electrical potentials, 14.6 and 17.3 millivolts respectively. The only ground for attributing a "specific chemical affinity for complement" to the immune antigen would be to compensate it for having a higher electrical potential than that possessed by the non-specific antigen. But De Kruif and Northrop (1) have pointed out that the electrical potential upon antigen particles plays only a lesser role in causing the adsorption of immune substances by the antigen; the adsorption is not *due to* the electrical charges on the antigen and immune body, but the effect on the electrical potential is the *result* of the adsorption. Accordingly these data indicate that fixation of complement is not in itself a specific immune reaction, but is due to certain physical characters of the antigen: that it matters not whether these physical properties are produced by means of an immune reaction or by simple non-specific reagents; and hence that a "specific chemical affinity for complement" on the part of antigen sensitized by immune serum either does not exist, or can at most be only a minor factor in producing complement fixation.

The non-specific reaction is of significance in the wider field of immunology, as an example of an immune reaction being duplicated by non-specific means. The particular conditions which have been used in this work to produce the non-specific reaction do not modify the cohesiveness and oil-wettability of the antigen to the same extent as would treatment with a strong immune serum; consequently the former reaction does not cause such powerful complement fixation as can be produced in the immune reaction. At present the writer is not aware of any clinical application of the phenomena which produce the non-specific reaction. But if other and more effective means are found by which the true immune reactions can be duplicated by simple non-specific reagents, therapeutic treatment of infectious diseases may be possible by these simpler methods, without resort to complex biological products such as anti-toxins and immune sera.

Summary

1. The mechanism of a non-specific complement fixation reaction is described.
2. The antigen in this non-specific reaction assumes physical properties which closely simulate those of an antigen sensitized by immune serum. This affords an example of a "specific immune reaction" being duplicated by simple non-specific means, *i.e.*, balanced physiological saline and normal guinea pig serum.

3. The data herein presented indicate that complement fixation is not in itself a specific immune reaction, but is produced by a modified antigen having certain physical properties, regardless of whether immune or non-specific means are used to produce these physical properties. Hence a specific chemical affinity for complement on the part of antigen sensitized by immune serum either does not exist, or at most can be of only minor significance in producing complement fixation.

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TYPES OF TUBERCLE BACILLI IN HUMAN TUBERCULOSIS¹

By R. M. PRICE²

Abstract

Four hundred and thirty-six cases of clinical tuberculosis were investigated with a view to ascertaining the types of the infecting organism. In this series of cases, 268 were children under 14 years of age, and 168 adults, 15 years and over. Both medical and surgical cases were studied. In the juvenile group, 230 patients proved to be infected with the human type, and 38, or 14.1%, with the bovine type of the tubercle bacillus. In the adult group 6, or 3.5%, proved to be infected with the bovine type, the remaining 162 cases with the human type of the tubercle bacillus. Bovine tuberculosis in man is milk-borne and preventable.

The determination of the types of tubercle bacilli in human tuberculosis has now covered a period of over five years. The technique used and other details in differentiation of the types as well as the results of our findings up to April 1, 1931 in 320 cases of tuberculosis, of which 220 were children under 14 years of age, and 100 adults 15 years and over, have already been published (1).

The lesions and the types of tubercle bacilli included in the previous communication are shown in Table I.

TABLE I

VARIETIES OF TUBERCULOSIS AND TYPES OF TUBERCLE BACILLI ENCOUNTERED IN 320 CASES OF TUBERCULOSIS INVESTIGATED PRIOR TO APRIL 1, 1931

Variety of tuberculosis	Children under 14 years of age		Adults, 15 years and over		
	Type of tubercle bacillus				
	Human	Bovine	Human	Bovine	Avian
Pulmonary tuberculosis	22	1	1		
Tuberculous meningitis	46	1	2		
Tuberculous pleurisy	2	0	11		
Bone and joint tuberculosis	72	3	14		
Renal tuberculosis	18	2	42		
Tuberculous adenitis	21	16	22		1
Tuberculosis of the tonsil	7	3	2		
Tuberculosis of the adenoid	4	1	0		
Tuberculous peritonitis	0	1	0	1	
Hodgkin's disease	0	0	1		
Multiple periarticular abscesses	0	0	0	*1	
Tuberculous bacillemia	0	0	2		
Lupus vulgaris	0	1	0		
Mesenteric tuberculosis	0	1	0		

*Attenuated.

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This study has been continued during the past year and, from April 1, 1931 until February 1, 1932, 116 additional strains of the tubercle bacillus have been isolated; 48 from tuberculous children under 14 years of age, and 68 from adults 15 years and over.

The method of isolation, cultivation and final differentiation of types were essentially the same as already described. The only change in the earlier technique was the addition of a modified Dorset's egg medium containing small amounts of soluble silica which, we had found, favored the growth and development of tubercle bacilli in the test tube (2).

The variety of tuberculosis and types of bacilli in these 116 cases are shown in Table II.

TABLE II

VARIETIES OF TUBERCULOSIS AND TYPES OF BACILLI ENCOUNTERED IN 116 CASES OF TUBERCULOSIS INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Children under 14 years of age		Adults, 15 years and over	
		Type of tubercle bacillus			
		Human	Bovine	Human	Bovine
Bone and joint tuberculosis	32	12	1	18	1
Tuberculous adenitis					
(a) Cervical	28	8	4	14	2
(b) Inguinal	1	0	1	0	0
Genito-urinary tuberculosis	13	0	0	13	0
Tuberculous meningitis	8	3	1	4	0
Pulmonary tuberculosis	14	10	0	4	0
Tuberculous pleurisy	9	2	0	7	0
Tuberculous peritonitis	5	0	0	5	0
Tuberculous tonsilitis	2	1	1	0	0
Tuberculous adenoid	2	1	1	0	0
Tuberculous bacillemia	1	1	0	0	0
Ischio-rectal abscess	1	1	0	0	0

The cases studied are divided into two age groups and for convenience are retabulated in Tables III and IV.

TABLE III

VARIETIES OF TUBERCULOSIS ENCOUNTERED IN 48 CASES IN CHILDREN, INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Variety of tuberculosis	No. of cases
Bone and joint tuberculosis	13	Tuberculous pleurisy	2
Tuberculous adenitis		Tuberculous tonsilitis	2
(a) Cervical	13	Tuberculosis of the adenoid	1
(b) Inguinal	1	Tuberculous bacillemia	1
Pulmonary tuberculosis	10	Ischio-rectal abscess	1
Tuberculous meningitis	4		

TABLE IV
VARIETIES OF TUBERCULOSIS ENCOUNTERED IN 68 CASES IN ADULTS,
INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Variety of tuberculosis	No. of cases
Bone and joint tuberculosis	19	Pulmonary tuberculosis	4
Tuberculous adenitis	16	Tuberculous meningitis	4
Genito-urinary tuberculosis	13	Tuberculous peritonitis	5
Tuberculous pleurisy	7		

Discussion of the Anatomical Distribution of the Lesions

1. Bone and Joint Tuberculosis

Thirty-two cases of bone and joint tuberculosis were studied. In 19 instances the patients were adults, 15 years and over, and 13 were children under 14. The original material was, for the most part, pus aspirated from abscesses which had formed a connection with the lesion in the bone or joint. Some was removed surgically from diseased bones or joints.

The distribution of the bone and joint lesions in the adult group is shown in Table V.

TABLE V
DISTRIBUTION OF BONE AND JOINT LESIONS FROM 68 CASES OF TUBERCULOSIS IN
ADULTS, INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Type of tubercle bacillus	
		Human	Bovine
Tuberculosis of the spine	6	5	1
Tuberculosis of the hip joint	6	6	0
Tuberculosis of the sternum	1	1	0
Tuberculosis of the knee joint	3	3	0
Tuberculosis of the wrist joint	2	2	0
Tuberculosis of the shoulder joint	1	1	0

In 18 instances in adults the infecting organism was of the human type, and in the majority of cases the lesion in the bone or joints was secondary to tuberculosis in the lung. In one instance only was the infecting organism of bovine type.

The latter case, having a bovine infection, was a girl 19 years of age, born in New Hamburg, Ontario, who was admitted to Freeport Sanatorium, Kitchener, Ontario, on June 19, 1931, with a clinical diagnosis of tuberculosis of the spine. Her illness began in January, 1931, with pain in the back. A small abscess appeared in the region of the sacro-iliac joint, and in April of the same year, an abscess appeared in the region of the sternum. In July, she developed signs of severe cervico-dorsal Pott's disease, and in September, marked paresis of the upper and lower extremities. The patient died on

September 25, less than nine months from the time of the onset of her last illness. There was no history of contact with pulmonary tuberculosis, and no evidence of pulmonary disease in the patient herself, either on physical or X-ray examination. Pus aspirated from one abscess in the region of the sacro-iliac joint produced generalized tuberculosis in guinea pigs and rabbits. A bovine strain of the tubercle bacillus of unusually high virulence was isolated from this source.

Thirteen cases of bone and joint tuberculosis were studied in the juvenile group of cases. The distribution of the lesions and the type of infection in the group are shown in Table VI.

TABLE VI
DISTRIBUTION OF BONE AND JOINT LESIONS, AND TYPE OF INFECTION IN THE JUVENILE GROUP INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Type of tubercle bacillus	
		Human	Bovine
Tuberculosis of the knee joint	4	4	0
Tuberculosis of the hip joint	3	2	1
Tuberculosis of the ankle joint	2	2	0
Tuberculosis of the spine	2	2	0
Tuberculosis of the mastoid	1	1	0
Tuberculosis of the right cuboid	1	1	0

In 12 cases from this group the infection proved to be due to the human type of tubercle bacillus. The majority of the cases gave a history of contact with open pulmonary tuberculosis. Physical examination and X-ray of chest, or both, revealed evidence of tracheo-bronchial or pulmonary involvement, the evidence pointing to the respiratory route of infection.

In only one of this group was the infection due to the bovine type. This patient, a child three years of age, was admitted to the Hospital for Sick Children, Toronto, from Kirkland Lake, Ontario, with a clinical diagnosis of tuberculosis of the right hip joint. There was no history of contact with open pulmonary tuberculosis, and no evidence of pulmonary involvement on either physical or X-ray examination, the evidence pointing to the alimentary route of infection. Enquiry revealed the fact that this child had always lived in Kirkland Lake. For the first two years, until 1930, she was fed on raw milk from a local dairy. Following a severe outbreak of septic sore throat in that community, pasteurization of milk was made compulsory; since then the child was fed sterilized milk. Granulation tissue removed from the hip joint proved highly infective for guinea pigs and rabbits, a typical bovine strain of the tubercle bacillus of high virulence being isolated.

2. *Tuberculosis Adenitis—Cervical and Inguinal*

Thirty-one cases of tuberculous adenitis were investigated. In 30 instances the cervical glands were involved and in one instance, the inguinal glands were affected. Fifteen occurred in children under 14 years of age, and 16 in adults, 15 years and over.

TABLE VII
DISTRIBUTION OF LESIONS AND TYPES OF INFECTION IN CASES OF TUBERCULOUS ADENITIS
INVESTIGATED FROM APRIL 1, 1931 TO FEBRUARY 1, 1932

Variety of tuberculosis	No. of cases	Children under 14 years of age		Adults, 15 years and over	
		Type of tubercle bacillus			
		Human	Bovine	Human	Bovine
Cervical	30	10	4	14	2
Inguinal	1	0	1	0	0

Adult Group

Analyzing the results obtained in the adult group, it will be noted that in 14 instances the infecting organism was of the human type and in two cases the infection was caused by the bovine type.

Case Reports

Case No. 1. Mr. W., age 16 years. Admitted to the Toronto General Hospital from Mount Dennis, Ontario, with a clinical diagnosis of tuberculous cervical adenitis. The patient was born in Mount Dennis, Ontario, and for seven years had lived in Stratford, Ontario. All his life this individual had been accustomed to drinking raw milk. Physical examination and X-ray of the chest gave negative results. Glands removed at operation proved tuberculous, a typical bovine strain of the tubercle bacillus being isolated.

Case No. II. Mr. S., age 36 years, mining engineer, admitted to the Private Patients' Pavilion, Toronto General Hospital, from Cobalt, Ontario, with a clinical diagnosis of tuberculous cervical adenitis of four months' duration. The patient was an unusually healthy adult. There was no evidence of disease in the chest, the infection being in the region of the glands of the neck, probably secondary to a tuberculous tonsilitis. The cervical glands, removed at operation, yielded a typical bovine strain of the tubercle bacillus. This patient is to return in a few months for tonsilectomy.

Upon enquiry it appeared that the patient had been in the habit of drinking large quantities of raw milk supplied by a local dairy.

Juvenile Group

In 14 instances in this group the infection was in the region of the cervical glands, and in one instance the inguinal glands were involved. In 9 of the 13 cases of cervical adenitis, the infection was of human type; in 4 cases the infecting organism proved to be of bovine type.

Case Reports

Case No. I. W. R., age 7 years. Admitted from Kirkland Lake, Ontario, with a clinical diagnosis of tuberculous cervical adenitis, 1½ years' duration. There was no history of contact with pulmonary tuberculosis, and no evidence of pulmonary tuberculosis on physical or X-ray examination.

This boy had always lived in Kirkland Lake, and had been fed on raw milk from a local dairy.

Case No. II. H. McB., age 9 years, admitted to the Hospital for Sick Children, from Waterloo, Ontario, with a clinical diagnosis of tuberculous cervical and submaxillary adenitis. There was no history of contact with pulmonary tuberculosis, and no evidence of tuberculosis either on physical or X-ray examination. This child had always lived in the country, and had always had raw milk.

Case No. III. D. M., age 8 years, admitted to the Hospital for Sick Children from Haileybury, Ontario, with a clinical diagnosis of tuberculous cervical adenitis. There was no history of contact with tuberculosis and no evidence of tuberculosis in the chest, either on physical or X-ray examination. This boy had always been fed on raw milk from various local dairies.

Case No. IV. T. G., age $5\frac{1}{2}$ years, admitted to the Hospital for Sick Children, from Copper Cliff, Ontario, with a clinical diagnosis of tuberculous cervical adenitis. There was no history of contact with pulmonary tuberculosis and no evidence of tuberculosis elsewhere, either on physical or X-ray examination. This boy was always fed on raw milk obtained from various local unpasteurized sources.

One case of tuberculosis of the inguinal glands was studied, in a child three years of age. Unfortunately no details were available in this case. A typical bovine strain of the tubercle bacillus was isolated.

With one possible exception, in this group of cases the tuberculous infection was primary in the cervical glands, *i.e.*, the infection was probably caused by tubercle bacilli which entered the cervical glands from the mucous membrane of the nose, throat, pharynx and tonsils. Wherever possible, in these cases of tuberculous cervical adenitis, an effort was made to obtain the tonsils and adenoid tissue for microscopic study and guinea pig inoculation. In two cases of this group, the writer was again successful in demonstrating microscopic foci of tuberculosis in the tonsils and adenoid, and she succeeded in infecting guinea pigs with this tissue.

3. Tuberculosis of the Meninges

Eight cases of tuberculous meningitis were studied. In all instances the cultures were isolated directly or indirectly from the cerebro-spinal fluid withdrawn during life. Four of the patients were children, and four adults. With the exception of one juvenile case, the tuberculous process in the meninges was a terminal one originating from a focus in the lung. The tubercle bacilli isolated in the adult group of cases proved, without exception, to be of human variety.

Of the four juvenile cases, three proved to be infected with the human and one with the bovine type. In all cases from whom the human type of the tubercle bacillus was isolated, there was a history of contact with pulmonary tuberculosis, and the process was a terminal one, originating from a focus in the lung.

In one instance the infecting organism was of bovine type. This patient, a child five years of age, was being treated for typhoid fever by a local physician in the east end of the city. The parents, not satisfied with the progress of the child, consulted another physician, who, upon examination, found evidence of meningal involvement, though tuberculosis was not suspected. There was no history of contact with tuberculosis, nor any evidence of tuberculosis in the lungs. The cerebro-spinal fluid, however, yielded tubercle bacilli which proved of typical bovine type. Upon enquiry it appeared that this child was at one time resident in Scarboro, and had been fed on raw milk supplied by a local dairy, from which source he undoubtedly contracted his infection. (The writer previously reported upon a small milk-borne epidemic of bovine tuberculosis in this district.)

4. Pulmonary Tuberculosis—Tuberculous Pleurisy

Twenty-three cases of intrathoracic tuberculosis were investigated. Twelve cases occurred in children, and 11 in adults. Of this group, 9 were cases of primary pleurisy with effusion; in 14 the disease occurred in the parenchyma of the lung.

In ten cases of the juvenile group the infection was found in the tracheo-bronchial glands, spreading out into the parenchyma of the lung, the organism being isolated from the sputum by means of the laryngeal swab. In two cases, the disease was in the pleura, the organism being isolated from the pleural effusion. Four adult cases of pulmonary tuberculosis were investigated. Without exception, the infecting organism in this group of cases was of the human type.

5. Genito-urinary Tuberculosis

Thirteen cases of genito-urinary tuberculosis were investigated, all in patients over 15 years of age. The disease affected the genital organs in three instances, and the urinary tract in ten. In every case studied the infecting organism proved to be of typical human type.

6. Tuberculous Peritonitis

Five cases of tuberculous peritonitis in the adult were investigated, of which four occurred in the female. The infecting organism in this group of patients was of human type.

7. Tuberculous Tonsillitis

Two cases of tuberculous tonsillitis were studied.

Case No. I. The patient, a boy, seven years of age, was suffering from a bilateral tuberculous cervical adenitis. The tonsils and adenoid removed at operation proved tuberculous on section and guinea pig inoculation. The glands were subsequently removed, and proved tuberculous. The human type of the tubercle bacillus was isolated from these several sources.

Case No. II. A patient, seven years of age, admitted to the Hospital for Sick Children, from Kirkland Lake, Ontario, with a clinical diagnosis of

tuberculous cervical adenitis. The tonsils and adenoid, removed at the same time as the glands, proved tuberculous on microscopic examination and guinea pig inoculation. A typical bovine strain of the tubercle bacillus was isolated from both tonsils, as well as from the adenoid and cervical glands.

8. *Tubercle Bacilli in the Blood Stream*

The tubercle bacillus was isolated from the blood stream of a child, five years of age, seen in the early stages of a tuberculous meningitis. The organism isolated from the blood and cerebro-spinal fluid, obtained at the same time, was of typical human type.

9. *Ischio-rectal Abscess*

One case of ischio-rectal abscess was studied. The patient, an infant eight months of age, was admitted to the Hospital for Sick Children, with a history of swelling in the right buttock of five weeks' duration. The abscess was evacuated, and scrapings from the abscess cavity produced extensive tuberculosis in the guinea pig, from which a typical human strain of tubercle bacillus was isolated.

It is of interest to note that the lungs of this patient were free from any evidence of tuberculosis. The tuberculin reaction however was strongly positive.

Summary

Summarizing the results obtained (Table VIII), it will be noted that there have been examined, by complete laboratory means, 436 strains of the tubercle bacillus, isolated from as many clinical cases of tuberculosis. Of this number, 268 patients were children under 14 years of age and 168 were adults, 15 years and over.

TABLE VIII
AN ANALYSIS OF THE ISOLATIONS OF TUBERCLE BACILLI FROM
VARIOUS HUMAN SOURCES

Variety of tuberculosis	No. of cases	Children under 14 years of age		Adults, 15 years and over	
		Type of tubercle bacillus			
		Human	Bovine	Human	Bovine
Bone and joint tuberculosis	121	84	4	32	1
Tuberculous adenitis	87	29	20	36	3
Genito-urinary tuberculosis	75	18	2	55	0
Tuberculous meningitis	58	49	2	6	0
Pulmonary tuberculosis	38	32	1	5	9
Tuberculous pleurisy	22	4	0	18	0
Tuberculous peritonitis	7	0	1	5	1
Tuberculous tonsilitis	14	8	4	2	0
Tuberculous adenoids	6	4	2	0	0
Tuberculous bacillemia	3	1	0	2	0
Ischio-rectal abscess	1	1	0	0	0
Hodgkin's disease	1	0	0	1	0
Multiple periarticular abscesses	1	0	0	0	*1
Lupus vulgaris	1	0	1	0	0
Mesenteric tuberculosis	1	0	1	0	0

*Attenuated.

Both Medical and Surgical Cases Studied

Analyzing the results obtained in the juvenile group of cases, it will be noted that in 230 patients the infection was caused by the human type of the tubercle bacillus and could, with very rare exception, be traced to a known source of exposure to open pulmonary tuberculosis. This group, too, in the majority of instances, showed evidence of tracheo-bronchial or parenchymatous involvement of the lung, the disease being respiratory in origin.

In 38 children the infection was caused by the bovine type of the tubercle bacillus. Enquiry in every instance revealed the fact that the child had been fed on raw milk while exposure to human pulmonary tuberculosis was usually denied or could not be traced. Physical examination, X-ray, or both, failed to show evidence of pulmonary involvement, the evidence pointing to the alimentary route of infection. Bovine tubercle bacilli have been isolated from the following regions: bones, joints, glands, tonsils, adenoid tissue, meninges and in one instance from the lung. Thus no region of the body appears immune to infection with the bovine type, by far the commonest site of infection being the glands at the side of the neck.

Of particular interest among the new cases of bovine tuberculosis encountered are three cases from Kirkland Lake, Ontario. Two of these came to Toronto prior to the outbreak of the epidemic of septic sore throat in that community, while the third case appeared shortly following the epidemic. Kirkland Lake, Ontario, a community of 8,000, was supplied with raw milk from four local dairies. In the latter part of December 1930, there occurred a severe outbreak of septic sore throat in that community, the source of this epidemic being finally traced to one dairy. It is interesting to note that seven head of cattle from the infected dairy were slaughtered and that, though these animals were presumably tuberculin negative and belonged to an accredited herd, one of the seven animals was so grossly infected with tuberculosis that the whole carcass was condemned. Subsequent to this epidemic the sale of raw milk was prohibited, and a compulsory pasteurization by-law was passed, but not until this disastrous experience did the community realize the importance of safe milk.

Similarly, earlier in the 1928 studies, the writer reported a milk-borne outbreak of tuberculosis in Scarboro, the infection being traced to one dairy distributing raw milk. Cases of apparently residual infection in that community have continued to arise from time to time, and, as recently as 1931, a case of tuberculous meningitis in a child five years of age was traced to that source.

Thus, in 14.1% of the cases of tuberculosis studied, the infection was caused by the bovine type of the tubercle bacillus. The importance of bovine tuberculosis in childhood lies not only in the incidence in which it exists, but also in the ease with which the disease, leading to disablement, disfigurement and entailing a great economic loss in a community, can be prevented.

The effective pasteurization of milk appears to be the only solution for the prevention of bovine infection. Pasteurization, however, can be considered

safe only when the procedure is carried out under rigid and scientific supervision.

The excellent results obtained in Toronto from the standpoint of eradication of bovine tuberculosis are attributable mainly to the efficient pasteurization of milk, and afford an unchallenged demonstration on the control of bovine tuberculosis in man.

Summarizing the results obtained in the adult group of cases investigated it will be noted that of the 168 cases of tuberculosis examined, 130 were suffering from so-called surgical tuberculosis and in 38 instances the disease was limited to lungs and pleurae.

In this series of cases the main attention of the writer was given to the determination of the type of tubercle bacillus involved in the lesion, no great effort being made to determine the portal of entry, nor to trace the course of the organism within the body. This was particularly true of the medical cases. On the other hand, in the so-called surgical group, the disease process, whether in the bone, joint or kidney, could usually be demonstrated as secondary to some latent or active tuberculous focus in the lung.

Of the 168 cases studied, 162 proved to be infected with the human type of the tubercle bacillus and 6 with the bovine type. In two of these latter cases, the organism was of attenuated virulence for experimental animals, while in the remaining four cases the bacilli were of standard virulence. The localization of the infection in this group was as shown in Table IX.

TABLE IX
SITE OF INFECTION IN SIX CASES OF TUBERCULOSIS DUE TO THE
BOVINE TYPE OF BACILLUS

Variety of tuberculosis	No. of cases	Variety of tuberculosis	No. of cases
Tuberculosis of the spine	1	Tuberculous peritonitis	1
Tuberculous cervical adenitis	3	Periarticular abscesses	1

In five of the six cases, the disease occurred in young adults, 16 to 24 years of age, the history suggesting the possibility of it being a residual childhood infection with the bovine tubercle bacillus which had become active during the stress and strain of adolescent and young adult life. In one instance, the disease was undoubtedly contracted in the third decade of life. It is also of interest to note that, as a result of the infection, two out of six cases died, the tuberculous process being acute and of short duration. The remaining four patients made an uneventful recovery.

This finding appears in striking contrast to the juvenile group. Here, although the morbidity rate is much higher than that encountered in adults, the mortality rate is relatively low, only two patients out of 38 succumbing to the infection.

Conclusions

1. Bovine infection was encountered in 38, or 14.1%, of 268 cases of tuberculosis in children under 14 years of age.
2. Bovine tubercle bacilli were isolated in 6, or 3.5%, of 168 adult patients studied.
3. Bovine tuberculosis in man is milk-borne, and preventable.

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THE INFLUENCE OF SILICA UPON THE GROWTH OF THE TUBERCLE BACILLUS¹

BY R. M. PRICE²

Abstract

The addition of small amounts of soluble silica to modified Dorset's egg medium has been noted to enhance the growth of the human type of the tubercle bacillus in the test tube. This is manifested by a lessening of the latent period of growth and a greater luxuriance of growth.

Introduction

Silicosis is an important industrial disease, affecting principally gold miners, granite cutters and occasionally cement and quarry workers. Compounds of silicon are very common, comprising about 28% of the earth's crust. The element manifests itself in two forms: silica or the oxide of silicon, which exists in crystalline or amorphous forms as in quartz, flint, sandstone, opal and diatomaceous earths; and silicates of which clay is an example.

Only workers exposed to certain rock dusts show a disposition to the development of silicosis. This is mainly due to the presence of crystalline silica, the dust of amorphous silica and the silicates being apparently innocuous.

In the lung, silica accumulates in the crystalline form, or in an invisible form designated "occult." The amount of fibrosis in the lung is in proportion to the amount of occult silica. It is believed that the fibrous reaction arises as a result of the silica particles undergoing a change from the visible to the occult form. The more readily this change occurs, the more serious is the tissue reaction.

Various hypotheses have been advanced as to the nature of the invisible silica. Watkins-Pitchford and Moir (6) have claimed to demonstrate that as high as 99% of particulate silica in the silicotic lung is invisible in ordinary histological preparations.

Gye and Purdy (4) have shown that colloidal silica is toxic when administered parenterally to experimental animals. This substance, silica sol, or orthosilicic acid, is derived from silica by the addition of two molecules of water. Gye and Kettle (2, 3) express the view that in the lung the inhaled silica is slowly transformed by the tissue juices into colloidal silica, either directly or through the intermediate form of sodium silicate.

Clinically, the majority of individuals suffering from silicosis ultimately succumb to pulmonary tuberculosis. In these individuals, the superimposed tuberculous process spreads with great rapidity, death ensuing in a comparatively short time. That silicosis should render the lung particularly vulnerable to tuberculosis has been the subject of much theorizing. Blocking of the lymphatic exits of the lung has been held responsible, but it is particularly interesting to note that the same reason is advanced to explain the opposite

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situation of the relative immunity of coal miners to tuberculosis. Mavrogordato (5) advanced the view that the defensive mechanism in the lung is broken down by fixation of numerous endothelial cells by silica dust, so that not a sufficient number of cells is left to combat tubercle invasion. Gardner (1) believes that phagocytes are attracted from a tuberculous focus to silica fragments in outlying regions and possibly spread the infection in that way. It is believed by others that susceptibility to silicosis is regularly associated with constitutional predisposition to tuberculosis. Avascularity of the silicotic nodule is another theory advanced.

All observers, however, are agreed upon the fact that the tuberculous infection invariably gains a stronghold in the silicotic nodules, or so-called silica nests. These foci evidently constitute the most vulnerable spot in the silicotic lung.

It suggested itself to the writer that silica might, in some manner, have increased the pathogenic effects of the tubercle bacillus *in vivo*, and she decided to observe the effect of certain silicon compounds upon the growth of the tubercle bacillus in the test tube.

Experimental

Materials and Method

Sodium silicate and silicic acid appeared to be the most logical compounds to use, and accordingly a solution of each was prepared, standardized to contain 1 mg. of silica per cc.

The method used was to add 1 cc. of the solution of either sodium silicate or silicic acid to every 100 cc. of a modified Dorset's egg medium. The medium was then prepared and sterilized in the usual manner. The tubes were seeded in the ordinary way, paraffined and incubated at 37.5° C., each strain so isolated being controlled with the usual differential media.

Results

In this manner, 81 initial cultures of the tubercle bacillus isolated directly from various human sources or indirectly through guinea pig inoculation were studied. The results obtained have been uniformly consistent, the presence of small amounts of sodium silicate or silicic acid in the culture media favoring the growth of the tubercle bacillus, as manifested by a lessening of the latent period of growth. Colonies became apparent to the naked eye in 6 to 14 days (the average time of appearance of the colony being 8 to 9 days), though colonies were observed as early as the fourth day in transfers. The colony on silica medium is small and dry, increases rapidly in size, assuming a warty, umbilicated appearance, and is fully three times the size of the control. Growth increases rapidly so that at the end of three weeks the amount of culture is about equal to that usually obtained in double that time.

Another constant feature is the production of pigment, all colonies showing an early golden-yellow coloration, richer and more distinct than that shown by the controls.

Of the 81 initial cultures studied, 51 strains were isolated through guinea pig inoculation, and 30 directly from various human sources, such as urine, sputum, cerebro-spinal fluid, and pus. In one instance in which the organism failed to grow on ordinary Dorset's medium it was isolated from ascitic fluid from a case of tuberculous peritonitis by this method. In the direct isolations, colonies became apparent between 8 and 14 days, and in one instance the growth was not only visible in 8 days, but was profuse and luxuriant, covering the whole surface of the slant.

All the direct cultural experiments were controlled by guinea pig inoculation. Where growth failed to appear on a medium containing silica, the biological test, without exception, proved negative.

The addition of larger quantities, namely 5 and 10%, of sodium silicate or silicic acid to the culture medium did not appreciably influence either the rate or the amount of growth of the bacillus, which may possibly be accounted for by an alteration in the pH of the culture medium. This, in the experience of the writer, is an important factor in the cultivation of this organism.

The writer is at the present time endeavoring to ascertain the effect of some other silicon compounds; namely, quartz suspension, aluminium silicate and diatomaceous earths. Preliminary tests tend to indicate that growth is enhanced by the addition of these substances in proportion to their solubility, the most stimulating effect being found on the addition of small quantities of sodium silicate, the least stimulation on the addition of quartz suspension. Thus it would appear that the presence of soluble silica in the culture medium renders this substance more readily diffusible, and therefore, more readily available for the metabolism of the organism. This is probably the explanation for the increased growth of the tubercle bacillus in these cultures.

Summary

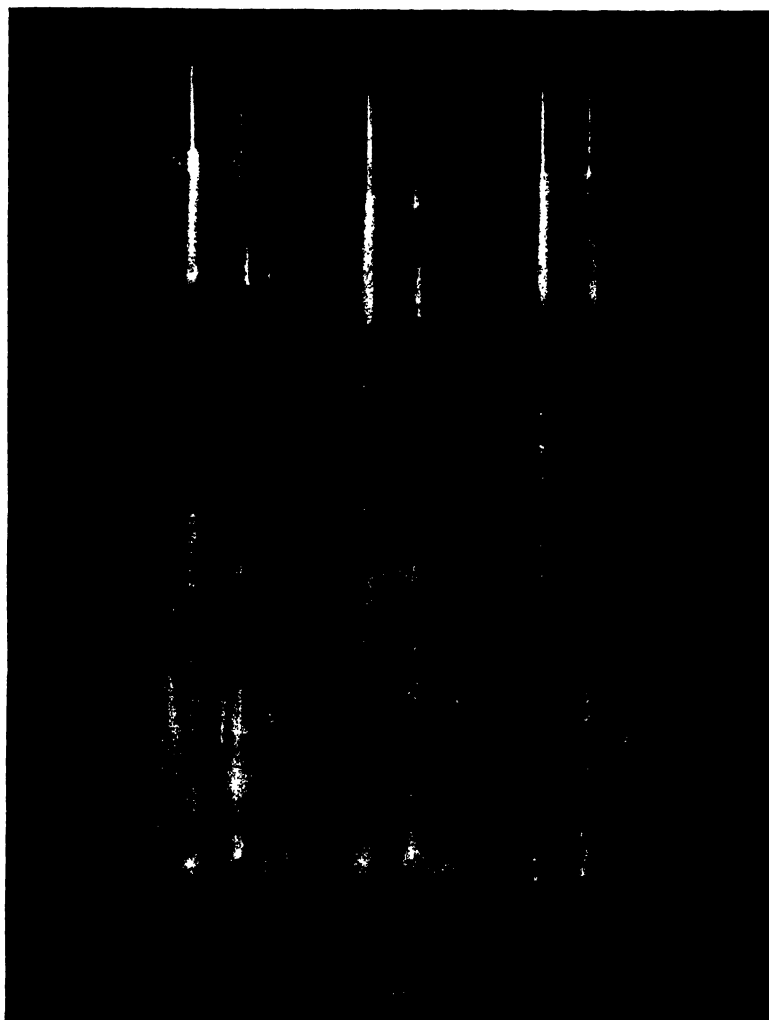
The writer has investigated *in vitro*, the effect of certain silicon compounds, namely, sodium silicate and silicic acid, upon the growth and development of the human type of the tubercle bacillus. Eighty-one initial cultures, isolated either directly or indirectly through guinea pig inoculation from various human sources, have been studied. Without exception the addition of 0.01 mg. of silica per cc. of medium enhanced the growth and development of the tubercle bacillus. This was manifested by a lessening of the latent period and a greater luxuriance of growth. This stimulating effect is continuous throughout the whole period of growth.

The results obtained with other silicon compounds now under investigation tend to indicate a similar effect, which appears to be in proportion to the solubility of the silicon compound. Thus it is found that the addition of small amounts of sodium silicate causes the greatest stimulation, and quartz suspension the least.

The stimulating effect of soluble forms of silicon on the tubercle bacillus *in vitro* suggests that some such factor may be involved in the rapidly progressive tuberculosis associated with silicosis.

TABLE I
GROWTH OF VARIOUS STRAINS OF TUBERCLE BACILLUS ON DIFFERENT MEDIA

No. of strain	Method of isolation	Amount of growth on sodium silicate media	Amount of growth on silicic acid media	Amount of growth on glycerol-egg media	Time of appearance of colony, days
H. 346	Indirect g. p.*	++++	+++	++	7-8
H. 350	Indirect g. p.	++++	++	±	9
T. 351	Indirect g. p.	+++++	+++	++	8
T. 353	Indirect g. p.	++++	+++	±	10
H. 359	Direct pus	+++	++	+	12-13
H. 360	Direct pus	+++	±	+	12
P. 361	Indirect	+++	+++	±	12
P. 363	Indirect	+++	++	+	10
H. 364	Indirect	++++	++	±	8
T. 366	Direct pus	++	+	±	8
T. 367	Direct pus	++	±	+	7
H. 368	Indirect g. p.	++++	+++	±	7
T. 369	Direct c. s. f.**	++	+	±	8
W. 370	Indirect	+++	±	±	12
W. 371	Indirect	+++	++	±	10
W. 372	Direct asc. f.	+++	±	No growth	14
W. 373	Direct pus	++	±	+	11
T. 374	Indirect	++++	+++	++	8
T. 375	Indirect	+++	++	±	8
T. 376	Indirect	+++	++	±	8
H. 378	Direct c. s. f.	++	±	±	10
H. 379	Indirect	++++	+++	++	8
T. 380	Direct urine	++++	±	++	8†
T. 381	Indirect g. p.	+++	±	±	8
T. 382	Direct pus	++	+	±	9
T. 383	Indirect g. p.	+++	±	±	9
H. 384	Indirect g. p.	++++	+++	++	10
H. 386	Indirect g. p.	+++	++	+	7
T. 387	Direct	++	±	± 1 colony	10
T. 388	Indirect	++++	±	±	9
W. 389	Indirect g. p.	++++	+++	±	9
W. 390	Indirect	++++	+++	++	7
T. 391	Indirect	+++	++	±	6
H. 392	Indirect g. p.	++++	+++	++	9
H. 393	Indirect	++++	±	++	7
H. 394	Direct c. s. f.	++	+	±	8
T. 395	Indirect	++++	+++	±	7
H. 396	Indirect	+++	++	+	7-8
H. 399	Indirect	++	++	+	8
H. 401	Indirect	++++	+++	±	7
H. 402	Indirect	++	++++	±	6-8
T. 403	Indirect	++++	+++	++	6-7
T. 404	Direct pus	+++	++	±	8
T. 407	Direct c. s. f.	++++	++	++	7
T. 409	Direct pus	++++	+++	++	9
T. 410	Indirect	++++	±	++	8
T. 414	Indirect	++++	+++	++	9
H. 415	Indirect	++++	+++	+	7
H. 416	Indirect	+++	++	±	8
H. 417	Indirect	++++	+++	+	9
H. 418	Direct pus	++++	+++	++	7-8
H. 419	Indirect	++++	++	+	9-10
H. 420	Indirect	++++	++	+	7
T. 421	Direct pus	++	+	±	8
T. 422	Indirect	+++	++	+	10
T. 423	Direct pus	++++	+++	+	10
T. 424	Indirect	++++	++	±	10
H. 425	Direct c. s. f.	++	+	±	11



*Strain No. H7. I, Glycerol-egg medium. II, Glycerol plus 1% of silicic acid.
III, Glycerol plus 1% of sodium silicate.*

TABLE I—*Continued*
GROWTH OF VARIOUS STRAINS OF TUBERCLE BACILLUS ON DIFFERENT MEDIA

No. of strain	Method of isolation	Amount of growth on sodium silicate media	Amount of growth on silicic acid media	Amount of growth on glycerol-egg media	Time of appearance of colony, days
T. 426	Indirect	++++	++	+	10
T. 428	Indirect	++++	+±	+	10
T. 429	Direct pus	++	+	±	12
T. 430	Direct pus	++++	++	+	10
T. 431	Indirect	+++	++	±	
T. 432	Direct pus	Few colonies	Few colonies	No growth	12
T. 434	Indirect	+++	++	±	10
T. 435	Indirect	++++	+++	+	8
T. 438	Indirect	++++	++	+	9
T. 439	Direct	++++	++	+±	8
H. 440	Indirect	++++	+++	++	8
H. 441	Indirect	++++	Not cultured	+	8
W. 442	Direct pus	+++	+±	±	10
H. 443	Indirect	+++	++	+	9
T. 449	Direct	++++	+++	++	7
T. 450	Direct urine	+++	++	+	8
T. 450A	Direct pus	++++	++±	+±	10
T. 451	Direct pus				
H. 453	Direct pus	Few colonies	Occasional colony	No growth	10
T. 454	Indirect pus	++++	+++	+±	7
H. 455	Indirect	++++	+++	+	7
H. 456	Direct pus	++++	+++	++	7††
T. 457	Indirect	++++	++	+	7

*Guinea pig. **Cerebro-spinal fluid. †Profuse luxuriant growth in 8 days. ††Profuse growth in 7 days.

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HODGKIN'S DISEASE AND THE TUBERCLE BACILLUS¹

By R. M. PRICE²

Abstract

Five cases of clinically and histologically typical Hodgkin's disease were investigated with a view to determining the possible association between the avian tubercle bacillus and this disease. The results obtained in this group of cases studied proved consistently negative from the standpoint of avian tubercle infection.

Introduction

This common and fatal disease was first described by Hodgkin (6) in 1832. Clinically the disease appears more like an infectious process than a primary disease of the blood-forming organs, while the gross character suggests the presence of an invasive tumor.

The disease is commonly found in young persons, and more frequently in men than women. It begins as a painless swelling of the superficial glands and progresses with gradual enlargement of these glands, a similar enlargement of glands in the thorax or abdomen, and usually an increase in the size of the spleen. Anemia appears, and may reach a profound degree. There may be evidence of mechanical pressure exerted by the enlarged glands upon important structures, producing edema of the face and other regions; on the trachea, leading to emphysema; upon the thoracic duct or its afferent tributaries, producing a chylous ascitis. One of these mechanical influences, or inter-current infection, or the anemia and cachexia produced by the disease finally leads to death.

The lesions, which are perhaps best studied in the lymph gland, consist of a proliferation of lymphoid cells, which is soon followed by the appearance of a coherent tissue formed of large, paler cells, with elongated, pale-staining nuclei. They lie in no particular order, but soon spread to replace the normal tissue of the whole gland, obliterating the distinction between lymph cords and sinuses. Among these cells are found much larger ones, being many times the size of a lymphocyte, and constituting the most characteristic feature of the lesion. Their protoplasm, except for scattered irregular stainable shreds, is clear, and their outline rather ragged. They contain two or more large lobed and indented nuclei, usually lying close together. Besides the lymphoid, epithelioid and these large cells, there are usually found many eosinophiles. Occasionally giant cells, containing many small nuclei arranged in a ring or horseshoe, are present. These resemble more closely those found in foreign-body giant cells than those of the tubercle. This cellular condition prevails in the earlier stages and is followed by scar formation throughout the whole gland, coincident with the disappearance of the cells. These changes invariably involve the whole gland, obliterating its architecture

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by replacing lymph cords and sinuses with a uniform cellular tissue. There is an attempt at regeneration, but the newly formed lymphoid tissue is soon transformed into the above-mentioned cellular tissue.

The glands first affected are usually those at the root of the neck just above the clavicle, where nodular masses resembling a rounded collar are formed. Those higher in the neck are then involved, followed occasionally by an involvement of the axillary and inguinal glands. In the interior of the body, the bronchial, peritracheal, and mediastinal glands are extensively involved, forming large masses, compressing the trachea and frequently impairing the heart action. Very often these masses of glands will invade neighboring tissue, the lung or the pleura.

In the abdomen, the retroperitoneal, peripancreatic and periportal glands are commonly involved, the latter sometimes becoming large enough to obstruct the common bile duct and produce jaundice. The spleen is invariably affected, sometimes with nodular swellings and diffuse infiltration of pulp, and occasionally with great enlargement. Foci are sometimes found in the bone marrow. Hodgkin's lesions have been known to invade the wall of blood vessels. On the whole they are limited principally to lymph glands.

The etiology of Hodgkin's disease is obscure. The disease has some characteristics of tumor growth, but the greater weight of evidence suggests an infection which involves simultaneously much of the lymphatic system. At the present time, four principal views are held. It is regarded as being: (1) a specific infective granuloma, (2) a tumor, (3) a transition between a granuloma and a tumor, (4) an atypical form of tuberculosis.

Carl Sternberg (10) in 1898 advanced the view that Hodgkin's disease was an atypical form of tuberculosis. In 1910, Fraenkel and Much (3, 4) using the antiformin method demonstrated a granular Gram-positive bacillus, which they regard as a non acid-fast tubercle bacillus, in a number of lymph nodes of Hodgkin's disease; they published further evidence in support of this view in 1923 (5).

Ewing (2, p. 375) has emphasized the close relation between tuberculosis and Hodgkin's disease, indicated by the similar granulomatous nature of the two processes, by the finding of acid-fast bacilli in some of the lesions, by the rarely positive atypical reaction produced in experimental animals, and by the not uncommon incidence of generalized tuberculosis as a terminal manifestation. Subsequent to the investigations of Fraenkel and Much, Ewing identified Gram-positive granules and frequently acid-fast rods in many of the lesions of Hodgkin's disease, further strengthening his theory that the tubercle bacillus plays an important part in its etiology.

L'Esperance (7), in 1929, reported the successful isolation of avian tubercle bacilli from two cases of Hodgkin's disease. In 1930 and 1931 she published the results of the bacteriological findings in two more cases (8, 9), one of Hodgkin's disease, and one of Pel-Epstein disease, and on the strength of her findings advanced the claim that the avian tubercle bacillus was the etiologic agent in at least some forms of the disease. Unfortunately L'Esperance's original work is open to criticism in as much as she failed to eliminate the

possibility of spontaneous tuberculosis among the birds used in her experiments. She also failed to identify the strains isolated, first as regards their ability to form tuberculin, but mainly as regards their pathogenicity for the guinea pig, rabbit and fowl. Furthermore, up to the present time, no account supporting the foregoing findings has appeared in the literature. Branch (1) failed to isolate tubercle bacilli from three typical cases of Hodgkin's disease. He further states that Van Ess had negative results in three cases of Hodgkin's disease; Aronson, of the Henry Phipps Institute, failed to isolate tubercle bacilli either culturally or by inoculation of chickens in two cases of Hodgkin's disease; and Shultz was unsuccessful in producing tuberculous lesions in fowl with typical Hodgkin's material.

Because of her interest in avian tuberculosis in man, and mainly because of the possible association of the avian tubercle bacillus with Hodgkin's disease, the writer undertook a study of this subject. Since 1930, she has studied histologically and bacteriologically 12 unusual cases of adenopathy, designated as atypical tuberculosis, avian tuberculosis, and atypical Hodgkin's disease, but in this communication, the writer will limit her report to five cases of clinically and histologically typical Hodgkin's disease.

Materials and Methods

In every case, the material for study was a lymph gland removed at operation. In four instances the gland was removed from the region of the neck, and in one from the inguinal region.

The tissue for study was divided into two parts. One part was fixed in 10% formalin for microscopic section, the other was ground up with sterile sand, emulsified in normal saline, and with this the following experimental animals were inoculated: (a) two normal guinea pigs; (b) two normal rabbits; (c) two to three young, tuberculin-negative fowl. The guinea pigs each received 0.5 to 1 cc. of the emulsion subcutaneously; the rabbits and fowl, 3 to 5 cc. intravenously.

In addition, the material from four of the five cases studied was cultured directly on plain and glycerol-egg medium.

The animals were carefully watched. Some of the guinea pigs and rabbits were sacrificed, while the fowl were allowed to die spontaneously, inasmuch as the incubation period of the avian tubercle bacillus in fowl is indefinite, varying from four to twelve months. The guinea pig is relatively immune to infection with the avian tubercle bacillus, whereas the rabbit is extremely susceptible, the animal dying in less than three weeks of a generalized infection, frequently without evidence of localization or tubercle formation. Fowl are naturally susceptible to this type of infection. The results of these analyses were negative, with the exception of one case in which a strain of the human type of tubercle bacillus was isolated from both inoculated guinea pigs.

Case Reports

Case I

Jack B., male, age 11 years. Admitted to the Hospital for Sick Children on October 24, 1929, complaining of swelling of gland in right side of neck, of

one year's duration. Physical examination revealed a diffuse swelling of the glands in right side of neck extending from behind the ramus of the mandible to about the level of thyroid cartilage below. This mass was made up of many smaller glands.

Operation. Removal of gland.

Microscopic findings. The general topography of the gland was altered. In places the capsule was thickened and running through the gland could be seen a few fibrous bands. One of these was quite wide, and in it could be seen a moderate degree of lymphocytic infiltration. The germinal centres were ill-defined and the bulk of the tissue had been replaced by cells which were rather larger than lymphocytes. Among these could be seen a few fairly large mononuclear cells and a very occasional one in which there were several nuclei. Scattered throughout were seen quite a number of cells undergoing mitosis.

Diagnosis. Hodgkin's granuloma.

Bacteriologic findings. Two guinea pigs, inoculated subcutaneously with an emulsion of the gland, died at intervals of two and three months respectively, and proved negative for tuberculosis. The rabbits survived four and four and a half months respectively, and were free from any evidence of tuberculosis at post mortem. The fowl died after seven and ten months respectively, and proved to be free from tuberculosis.

Case II

Cecil R., male, age 6 years. Admitted to the Hospital for Sick Children on Sept. 3, 1930, complaining of swelling of glands in neck for the past two years. Tonsils and adenoids were removed in 1929, without any influence upon the glands in the neck. Physical examination revealed the cervical glands, particularly posterior left group, markedly enlarged. Axillary glands large and firm. Inguinal glands slightly enlarged. Liver, three fingers' breadth below costal margin; spleen enlarged to level of iliac crest.

Clinical diagnosis. Hodgkin's disease.

Operation. Excision of glands in left side of neck.

Microscopic findings. The general topography of the gland had been greatly altered, the great bulk of the lymphoid tissue had been replaced by cells which included endothelial cells, proliferating fibroblasts and some small mononuclear cells. The germinal centres were very indistinct. Amongst the various types of cells could be seen fairly numerous mitotic figures, also occasional eosinophiles.

Diagnosis. Hodgkin's granuloma.

Bacteriologic findings. Two guinea pigs died three and four months after inoculation and proved negative for tuberculosis. The rabbits died at intervals of six weeks and four months respectively, and proved to be free from tuberculosis. The fowl died four and seven months after inoculation, and proved to be free from any evidence of tuberculosis, gross or microscopic.

Case III

Mr. S., age 38 years, admitted to the Wellesley Hospital complaining of swelling of glands in both sides of neck, of eight months' duration. Physical

examination revealed extensive bilateral swelling of glands of the neck. Axillary glands enlarged and firm, otherwise examination negative.

Clinical diagnosis. Hodgkin's disease.

Operation. Excision of gland right side of neck.

Microscopic findings. The general structure of glands was greatly altered. Much of the lymphoid tissue had been replaced by endothelial cells. Scattered throughout were large cells with pale-staining cytoplasm, and large vesicular nuclei.

Pathological diagnosis. Hodgkin's granuloma.

Bacteriologic findings. Two of the inoculated guinea pigs died at intervals of six and seven months, and proved to be free from tuberculosis. Two of the rabbits were killed at three and three and a half months respectively, and proved to be free from tuberculosis. Of the two inoculated chickens, one died five, and the other nine months later, and proved to be free from any evidence of tuberculosis.

Case IV

Mr. G., age 42 years, admitted to Christie Street Hospital, with a history of swelling of glands in neck, axillae and groin, of two years' duration. Physical examination revealed moderately extensive involvement of glands in both sides of neck, axillae and groins. Epitrochlears were palpable. The spleen was enlarged and nodular. The liver was palpable.

Clinical diagnosis. Hodgkin's disease.

Operation. Excision of inguinal gland.

Microscopic findings. The general topography of the gland had disappeared. There was considerable increase in the connective tissue of the capsule. In some areas the bulk of lymphoid tissue had been replaced by proliferating endothelial cells. Occasional typical Sternberg cells were seen. In a few places there was a small amount of eosinophilic infiltration.

Pathological diagnosis. Hodgkin's disease.

Bacteriologic findings. Two guinea pigs inoculated with an emulsion of this gland were killed and autopsied six weeks later, and showed signs of generalized tuberculosis, from which source a typical human strain of the tubercle bacillus was isolated. The rabbits which were killed at intervals of two and five months respectively, proved to be free from any evidence of tuberculosis. Two tuberculin-negative chickens, inoculated intravenously with an emulsion from this gland, died at intervals of two and eleven months respectively and proved to be free from any evidence of tuberculosis.

Case V

Mrs. E. L., age 19 years, admitted to the Toronto General Hospital in July 1931, complaining of swelling of both sides of neck, of 13 months' duration. Physical examination revealed on left side of neck numerous palpable glands, which were firm, discrete and not tender, varying in size from that of a walnut to that of a pea. They were situated immediately above the clavicle, posterior to the sterno-cleido-mastoid. There were two large glands just above the

clavicle and smaller ones scattered above that. Palpable glands present on the right side on the same situation, but fewer in number.

Clinical diagnosis. Hodgkin's disease.

Operation. Excision of glands in left side of neck.

Microscopic findings. Sections showed the normal topography of the gland to be completely destroyed. Broad bands of fibrous tissue surrounded discrete islands of cells, among the latter being lymphocytes, large pale endothelial cells, frequent mononuclear giant cells and eosinophiles, and occasionally a multinuclear giant cell. Occasional lymphocytes were scattered throughout the fibrous tissue, also numerous eosinophiles.

Bacteriologic findings. Two guinea pigs inoculated subcutaneously with an emulsion from these glands were sacrificed two and six months after inoculation, and proved to be free from tuberculosis. Two rabbits, killed at two and four months respectively, were free from any evidence of tuberculosis. One chicken died two weeks later of an intercurrent infection, and the second bird died four months later, and showed no evidence of tuberculosis, gross or microscopic.

Summary and Conclusions

Five cases of clinically and histologically typical Hodgkin's disease were investigated bacteriologically. In four of the cases studied the disease occurred in males, in one instance in a female. The youngest patient in the group was a boy, six years of age, the oldest, a man, forty-two years of age. The duration of the disease varied from 13 months to 2 years before material was submitted for study. In two of the cases studied the disease was limited, as far as could be ascertained, to the lymph glands. Two of the patients showed marked splenic and hepatic enlargement. In four instances, the gland tissue failed to produce any evidence of tuberculous infection, gross or microscopic, in guinea pigs, rabbits or normal fowl.

In one instance, an inguinal gland, which histologically was typical of Hodgkin's granuloma, proved infective for the guinea pig. A typical human strain of the tubercle bacillus was isolated in culture. The isolation of a human strain of tubercle bacillus from Hodgkin's granuloma is interesting, but not unique.

In no instance was the tubercle bacillus isolated directly in culture from Hodgkin's material.

Up to the present time the writer has failed to substantiate the possible association between the avian tubercle bacillus and Hodgkin's disease.

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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES

XLIII. CYCLIC ACETAL AND KETAL FORMATION FROM α -PHENYL GLYCEROL AS FURTHER EXAMPLES OF THE "RING PARTITION PRINCIPLE"¹

BY MURIEL E. PLATT² AND HAROLD HIBBERT³

Abstract

The work represents an extension of the "Hibbert-Michael ring partition theory" to the interaction of α -phenyl glycerol with acetone and *p*-nitrobenzaldehyde respectively. The α -phenyl glycerol was prepared in the form of a pure crystalline product from cinnamyl alcohol and on condensation with acetone yielded the two expected, isomeric, five-membered (dioxolane) ring compounds. These two isopropylidene derivatives were isolated and their structures definitely determined by the usual hydrolysis and methylation technique. In this manner the corresponding α - and γ -methyl ethers of α -phenyl glycerol were isolated, the identity of which had been previously settled by direct synthesis employing well-established reactions.

The β -methyl ether of α -phenyl glycerol was obtained by methylating crystalline 1:3 *p*-nitrobenzylidene α -phenyl glycerol and then hydrolyzing the ether.

Condensation of *p*-nitrobenzaldehyde with α -phenyl glycerol should yield, according to the "ring partition principle", one six- and two five-membered cyclic acetals.

The crystalline isomer separating from the crude condensation product was shown to be the six-membered cyclic acetal. Removal of this left a viscous oil containing the five-membered acetals which, on methylation, and subsequent hydrolysis, yielded a small amount of the γ -methyl ether of α -phenyl glycerol, thus indicating the presence of some 1:2 *p*-nitrobenzylidene glycerol in the original reaction product. Due to the large number of theoretically possible five-membered rings it was not found possible to isolate, or prove the presence of, both five-membered acetals in the oil left after removal of the crystalline six-membered acetal. Presumably both of the structural five-membered acetals were formed, but due to their mutual solubility relations it was not possible to bring about a separation of crystalline forms.

Introduction

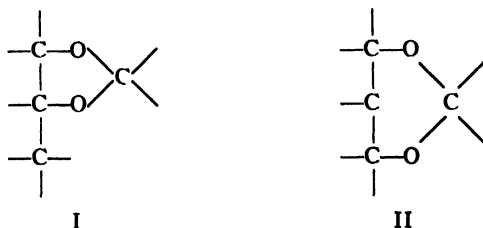
In the earlier work relating to the synthesis of cyclic acetals and ketals of various carbohydrates and polysaccharides carried out by Fischer (2, 3) and by Irvine (17), it was tacitly assumed, though without any proof, that such products as benzylidene glucose, isopropylidene glucose, benzylidene glycerol, etc., all possessed a five-membered ring configuration. Hibbert and Hill, some ten years ago, first pointed out (9, p. 748) that such assumptions were not necessarily true and in conjunction with various coworkers were able to show that, during the condensation of an aldehyde with a polyhydroxy alcohol, as glycerol, a "ring partition" occurs (7, 8, 11, 12, 14, 15) giving a reaction product consisting of a mixture of a five- and a six-membered cyclic acetal (I and II).

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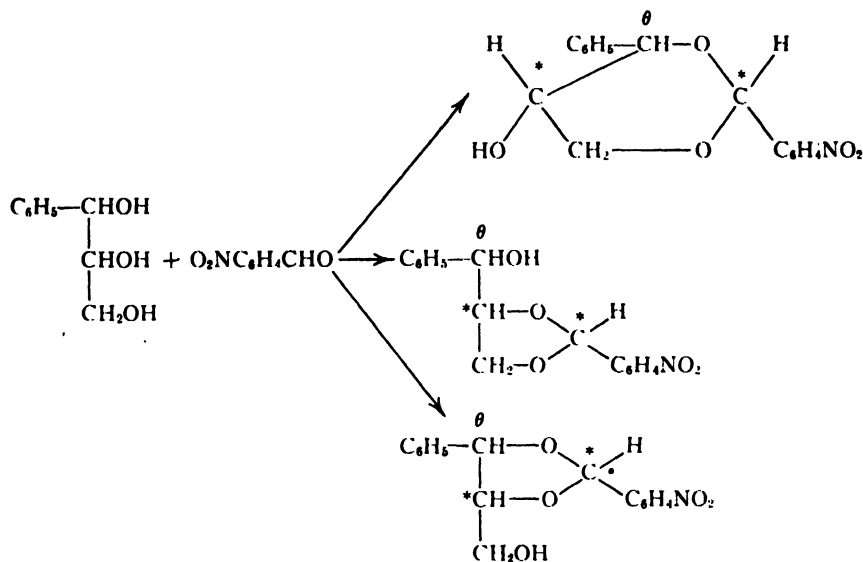
For the study of the formation and properties of cyclic acetals as a means of further confirming the theories of ring formation and the partition principle, glycerol was chosen as the most suitable polyalcohol. The various theories dealing with ring closure in organic compounds emphasize the ease of formation of both five- and six-membered rings. Glycerol contains both primary and secondary alcoholic groups and, just as in the case of the sugars, presents the opportunity for the formation of both five- and six-membered rings when condensed with a carbonyl compound. Due to the symmetry of the molecule difficulties due to optical isomerism, such as are found in the sugars, are not introduced. As the facts concerning the behavior of cyclic acetals accumulated, it was thought that the use of a substituted glycerol might give results which would be of interest, since a substituted glycerol is still more closely related to the sugars than is glycerol itself. An opportunity would also be afforded to test out the validity of the ring partition principle in a still more complex case. α -Phenyl glycerol was therefore chosen for this work and an attempt made to isolate all of the theoretically possible structural isomers obtained on condensing this alcohol with *p*-nitrobenzaldehyde and with acetone.

The presence of the phenyl group in the α -position of phenyl glycerol makes possible the formation of three structural isomers during the condensation of phenyl glycerol with *p*-nitrobenzaldehyde. Due to the rigidity of the ring, each of these in turn may exist as geometrical isomers (8) and the presence of the two asymmetric carbon atoms (*) indicates that each isomer must exist as optically resolvable, racemic forms. Further modifications might also be expected from the asymmetric carbon atoms (θ), in each of the structural isomers. This complexity may be seen by a glance at the diagram on page 631, in which the possibilities for structural ring isomerism are indicated.

As a means of identifying the possible structural isomers formed during the condensation of α -phenyl glycerol with acetone and *p*-nitrobenzaldehyde, it was necessary to prepare derivatives of α -phenyl glycerol by such methods that the position of the group introduced was known, and then to use these as reference compounds in identifying the products formed in the actual condensation. The methyl ethers of α -phenyl glycerol were chosen as the most satisfactory reference compounds, since it has been shown by Hibbert and Whelen (13) that the methyl group does not migrate during hydrolysis reactions with this type of compound.

The γ -methyl ether of α -phenyl glycerol (1-phenyl glycerol-3-methyl ether) was prepared by the methylation of cinnamyl alcohol with silver oxide and methyl iodide followed by oxidation of the double bond with dilute potassium

permanganate. This method, as stated by Fairbourne and Foster (1), yields a product of a high degree of purity, and it was possible to prepare a crystalline methyl ether melting at 54°C . The α -methyl ether of α -phenyl glycerol was prepared by methylating 1:1 phenyl vinyl carbinol, obtained by the interaction of phenyl magnesium bromide with acrolein, and oxidizing the resulting methyl ether with dilute potassium permanganate. This compound was also crystalline and has a melting point of 68°C .



The α -phenyl glycerol used in this work was prepared by treating cinnamyl alcohol with benzoylhydroperoxide and hydrolyzing the resulting glycidol with $0.02\text{ }N$ hydrochloric acid at 0°C . Moureu and Gallagher (18) had previously obtained phenyl glycerol as a colorless, very viscous oil distilling at $185\text{--}186^{\circ}\text{C}/5\text{ mm}$. but were unable to obtain it in a crystalline form. It was found that the α -phenyl glycerol prepared as above crystallized on standing at room temperature, the crystals melting at $98\text{--}99^{\circ}\text{C}$.

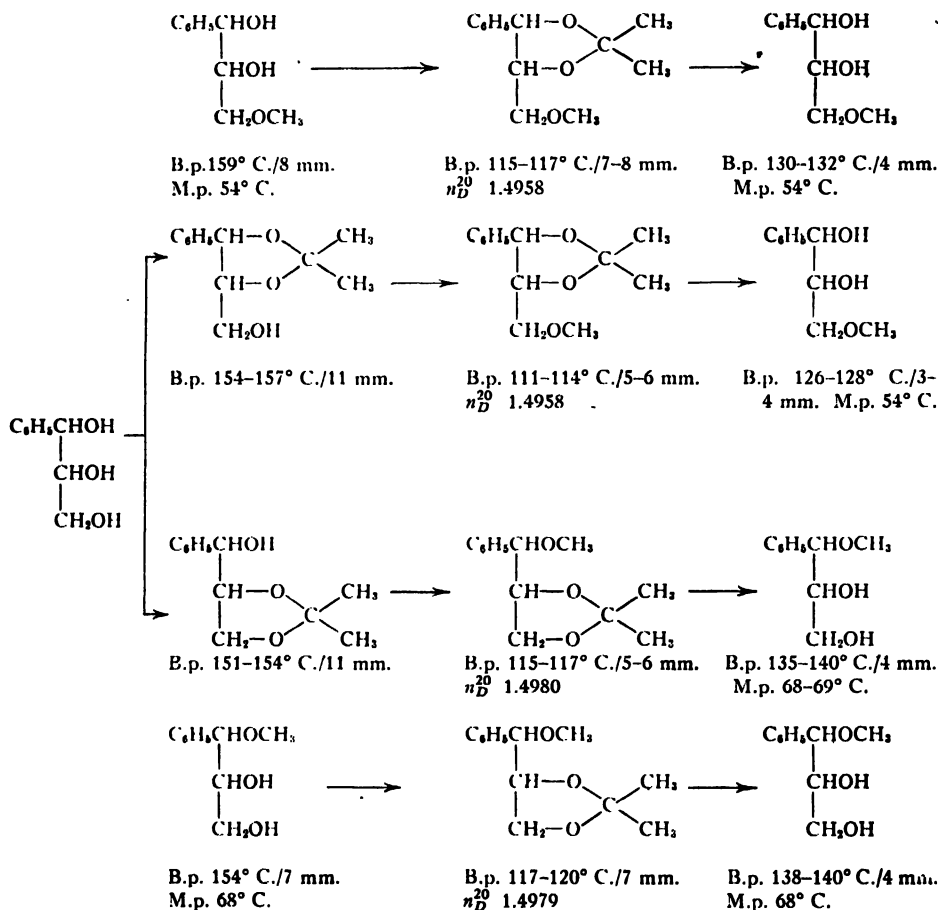
Condensation of α -Phenyl Glycerol with Acetone

It has been shown that the condensation of glycerol with acetone gives only a five-membered ring (16), all attempts to isolate the six-membered isomer having failed (10). It was, therefore, of interest to determine whether a "ring partition" occurs when acetone is condensed with α -phenyl glycerol, there existing the possibility for the formation of two isomeric five-membered rings due to the presence of the phenyl group in the α -position.

The condensation of α -phenyl glycerol with acetone was carried out in the presence of anhydrous copper sulphate in order to eliminate the possibility of ring migration, which would occur in an acid medium. The isopropylidene α -phenyl glycerol thus obtained was methylated and, after fractional distillation, the products compared with those resulting from the condensation of the α - and γ -methyl ethers of α -phenyl glycerol with acetone. On hydrolysis the

corresponding methyl ethers of α -phenyl glycerol were obtained, indicating that during the condensation a partition had occurred with the formation of the two isomeric five-membered isopropylidene α -phenyl glycerols. The ratio of the crystalline α -methyl ether to the crystalline γ -methyl ether (obtained by hydrolysis of the methylated isopropylidene phenyl glycerols) was found to be approximately 3:2. (This ratio is only very approximate, due to the difficulty of separating completely the structural isomers.) The relation of these derivatives to one another and to the two reference α -phenyl glycerol methyl ethers is indicated in Table I.

TABLE I

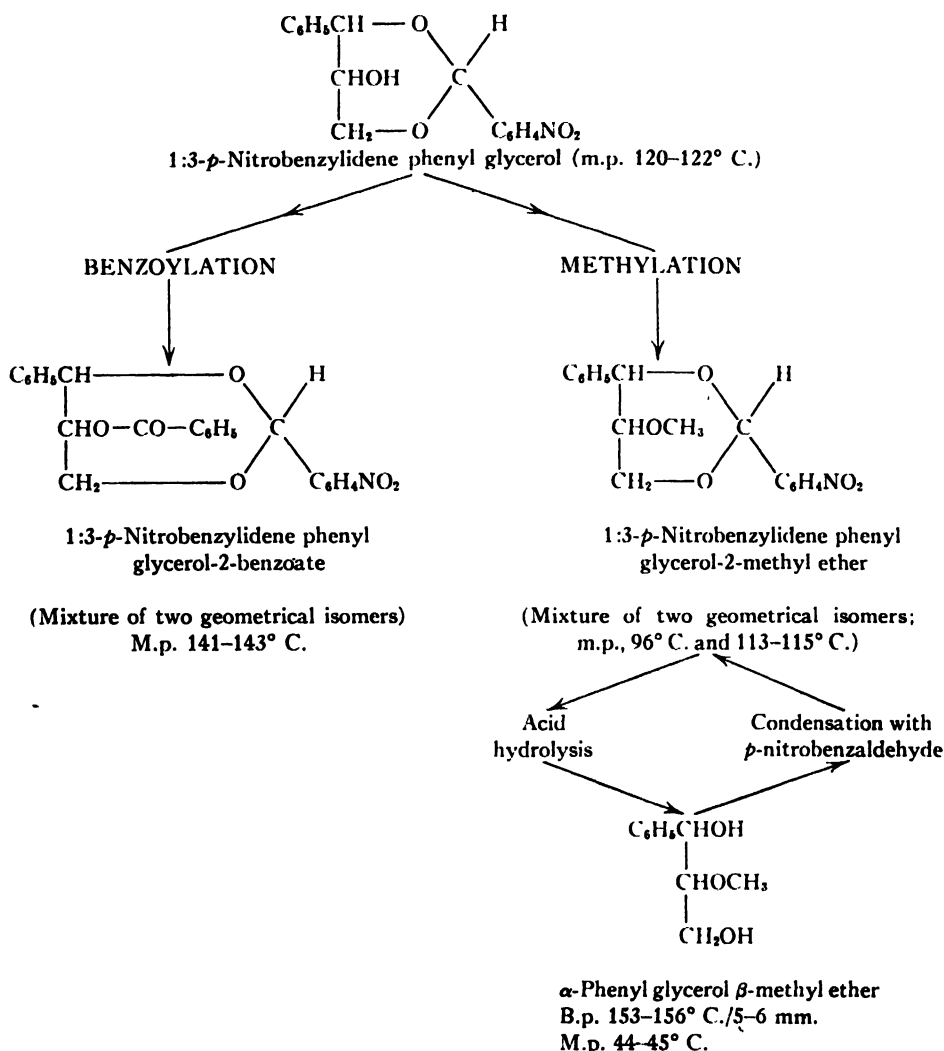


Condensation of α -Phenyl Glycerol with *p*-Nitrobenzaldehyde

According to the ring partition principle three structural isomers are to be expected from the condensation of *p*-nitrobenzaldehyde with α -phenyl glycerol; namely, two five-membered rings and one six-membered ring. By analogy with *p*-nitrobenzylidene glycerol it was to be expected that the six-membered ring isomer would prove to be a crystalline product and this was found to be

the case. It crystallized very slowly from a benzene-petroleum ether solution at -12°C . in the form of rosettes of fine yellowish needles. Repeated recrystallization did not give a sharp melting point (m.p., 120 – 122°C .) and, after standing at room temperature for a week, the melting point became even more

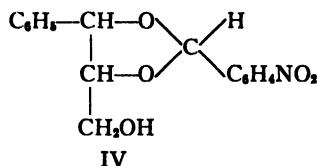
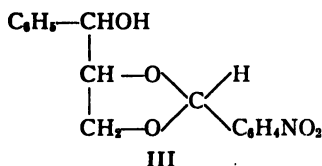
TABLE II



indefinite (m.p., 120 – 127°C .). Failure to obtain a sharp melting point is probably to be attributed to the spontaneous transformation of one geometrical isomer into the other, as has been observed with the six-membered *p*-nitrobenzylidene glycerol (8), and similar to the known transformation of the *cis*- into the *trans*- form of dibenzoyl ethylene (19). All efforts to separate this solid product into two different substances, one of which might have been one of the

five-membered ring forms, failed. Recrystallization resulted in a practically quantitative recovery of solid melting at 120–122° C. Methylation of the six-membered isomer, followed by acid hydrolysis, gave the β -methyl ether of phenyl glycerol (m.p., 44–45° C.). No indication was found of the α -methyl ether (m.p., 68° C.) or the γ -methyl ether (m.p., 54° C.) which would have been expected if the crystalline material obtained had been a mixture of the six-membered isomer with one or both of the five-membered forms. The proof of the structure of the crystalline 1:3-*p*-nitrobenzylidene phenyl glycerol is given in Table II.

The very viscous oily residue, which remained after the crystalline isomer had been removed as completely as possible, and which formed the greater part of the product from each condensation, is, according to the "ring partition principle", presumably a mixture of the two five-membered ring isomers (III and IV).

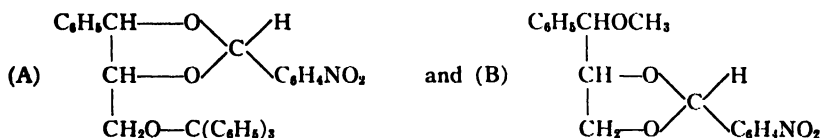


In an attempt to separate these two isomers, two methods were applied: (1) fractional distillation and fractional precipitation by means of organic solvents, and (2) preparation of a solid derivative.

The attempt to fractionally distil the oil resulted in decomposition of the product at 250° C. in spite of the low pressure used (0.01 mm.). The numerous attempts made to fractionally precipitate one of the isomers by means of various solvents were unsuccessful. Neither of the isomers was obtained in a crystalline form on cooling to –40° C.; the oil itself set to a hard glassy mass and a solution of the oil merely gave a heavy sludge from which, on filtration, no crystals could be obtained.

In the attempt to prepare solid derivatives from the crude mixture, attention was first directed to the methylation of the mixture of five-membered rings. As identification compounds for these two cyclic acetal methyl ethers, the α - and γ -methyl ethers of phenyl glycerol were condensed with *p*-nitrobenzaldehyde. No difficulty was experienced in obtaining a crystalline product from the condensation with the α -methyl ether and the two expected geometrical isomers were separated (m.p., 79–80° C. and 57–58° C.). The condensation of the γ -methyl ether with *p*-nitrobenzaldehyde, however, gave a dark red, viscous oil which resisted all efforts to obtain it in a crystalline form. An attempt was made to obtain the product in a pure state by distillation at low pressure but this brought about decomposition of the oil. It was hoped, therefore, that methylation of the oil obtained from the condensation of phenyl glycerol with *p*-nitrobenzaldehyde, followed by seeding with a crystal of the solid identification compound, would lead to a separation of the two five-membered rings. No solid could be obtained from the viscous oil, however, under any condition used.

The oily mixture of five-membered isomers was then subjected to acetylation, benzoylation, and *m*-brombenzoylation respectively, in an effort to prepare crystalline acetates, benzoates or *m*-brombenzoates, but without success. Trityl chloride, in presence of pyridine, reacts preferentially with primary hydroxyl groups when added to a compound which contains both primary and secondary hydroxyl groups (5). The isomer (IV) contains a primary hydroxyl group, whereas the isomer (III) contains a secondary hydroxyl group. On treatment with trityl chloride (IV) should form a trityl derivative while, under the same conditions, (III) would not. Trityl chloride, in presence of pyridine, was added to the crude acetal mixture in equimolecular proportions, according to the method described by Helferich (4), but no crystalline trityl derivative was obtained. The crude mixture was then subjected to methylation by means of silver oxide and methyl iodide, in the expectation that a mixture of two solid derivatives might be obtained, namely:



which might be expected to differ considerably in their solubility relations. This mixture was kept under varying conditions of temperature, in different solvents, for a period of over three months but there was no indication of the separation of any crystalline material.

The oily mixture of acetal methyl ethers was then subjected to acid hydrolysis in an attempt to isolate the α - and γ -methyl ethers of phenyl glycerol. It was found that hydrolysis did not take place as readily with the *p*-nitrobenzylidene α -phenyl glycerols as with the *p*-nitrobenzylidene glycerols. A small amount of oil was obtained with a boiling point corresponding very closely to the boiling point of the expected methyl ethers. From this oil it was possible to separate a few crystals of the γ -methyl ether of phenyl glycerol (m.p., 52–54° C.) but no crystals of the higher melting isomer (m.p., 68° C.) were obtained. This same difficulty was encountered with the methyl ethers obtained from the isopropylidene phenyl glycerols. Crystals separated from the oily mixture of the two isomers only after cooling to –12° C. for several weeks. The γ -methyl ether crystallized much more readily from the mixture than the α -methyl ether, the latter crystallizing out only when quite pure.

The difficulty experienced in obtaining the crystalline form of these methyl ethers from a mixture of the two isomers is explainable, in all probability, by the fact that isomeric forms are possible. Each of the methyl ethers contains two asymmetric carbon atoms (*).



There is thus the possibility of the formation of two isomeric inactive mixtures in the case of each methyl ether with the result that crystals form very slowly, if at all, in the mixture.

It has already been pointed out that each of the structural isomers formed during the condensation of α -phenyl glycerol with *p*-nitrobenzaldehyde is capable of existing in several geometrical and optical forms. The presence of these various isomers renders crystallization extremely difficult. In the work with sugars, for example, it has often been impossible to obtain a sugar or its derivative in the crystalline state if a trace of impurity is present. These various isomeric forms are also so similar that they serve as mutual solvents for each other and thus prevent crystallization. This difficulty was encountered at all stages in this work with the result that the derivatives prepared, although they should have been capable of isolation as solids—by analogy with the corresponding *p*-nitrobenzylidene glycerol compounds—persisted as viscous oils which could not be induced to crystallize.

Experimental

Preparation of α -Phenyl Glycerol

A chloroform solution of benzoylhydroperoxide containing 4.69 gm. of active oxygen, prepared by the method revised by Hibbert and Burt (6), was mixed at 0° C. with 37 gm. of cinnamyl alcohol and kept at 0° C. for 24 hr. with frequent shaking during the first hour. The benzoic acid formed during the reaction was removed by shaking the solution with an excess of 10% sodium hydroxide, the alkali removed by washing with water and the chloroform solution dried over anhydrous sodium sulphate. After removal of the chloroform, the product was fractionated under reduced pressure. α -Phenyl glycidol (34.3 gm.) was obtained boiling at 136–137° C./5 mm.; yield, 82.8%. On cooling to 0° C. the oil solidified to a mass of wax-like crystals; m.p., 26.5° C. Analysis:— Calcd. for $C_9H_{10}O_2$: C, 72.00; H, 6.66%. Found: C, 71.89; H, 6.58%. Mol. wt.: Calcd. for $C_9H_{10}O_2$, 150. Found (in $C_2H_4Br_2$); 155.2.

The above glycidol was treated at 0° C. with ten times its volume of 0.02 *N* hydrochloric acid and stirred constantly for six hours. After neutralizing the acid by shaking with silver carbonate, the solution was filtered and evaporated and the remaining oil distilled under reduced pressure, giving 32.6 gm. of α -phenyl glycerol; b.p., 184–186° C./5 mm.; yield, 84.9%. α -Phenyl glycerol is a colorless, odorless, hygroscopic, very viscous oil, soluble in water and alcohol. On standing at room temperature it solidified. Recrystallization from alcohol gave colorless crystals; m.p., 98–99° C. Analysis:— Calcd. for $C_9H_{12}O_3$: C, 64.28; H, 7.14%. Found: C, 64.15, 64.08; H, 6.95, 7.03%.

PREPARATION OF THE METHYL ETHERS OF α -PHENYL GLYCEROL

Preparation of the γ -Methyl Ether, $C_6H_5.CHOH.CHOH.CH_2OCH_3$

Methyl iodide (71 gm., twice the theoretical amount) was added to an ether solution of 35 gm. of cinnamyl alcohol in a three-necked flask fitted with an electric stirrer and a reflux condenser. To this mixture, which was constantly stirred, was added 60 gm. of silver oxide (twice the theoretical amount) in five portions at intervals of 30 min. Just enough heat was applied to keep the methyl iodide gently refluxing. At the end of six hours the reaction mixture was extracted several times with ether, the extract dried over potassium

carbonate and the ether removed by distillation. On fractionating the residue, 31.2 gm. of the γ -methyl ether of cinnamyl alcohol was obtained; b.p., 94–96° C./8 mm.; yield, 81.0%. Analysis:— Calcd. for $C_{10}H_{12}O$: C, 81.08; H, 8.10%. Found: C, 81.10; H, 8.00%.

Following the method of Fairbourne and Foster (1), 1800 cc. of an aqueous 1% solution of potassium permanganate was added at room temperature, to 11 grams of the above methyl ether dissolved in 2200 cc. of acetone. After standing for 4½ hours, the hydrated manganese dioxide was removed by filtration, the filtrate carefully neutralized with dilute hydrochloric acid and the acetone distilled off. The aqueous solution was evaporated on a water bath to 150 cc. and then fractionated. At 159° C./8 mm., 2.8 gm. of a pale yellow viscous oil distilled over which crystallized in yellow needles in the condenser (yield, 20.7%). On recrystallization, colorless crystals were obtained which melted at 54° C. The crystals are soluble in cold ether, benzene, alcohol and water; insoluble in petroleum ether. Analysis:— Calcd. for $C_{10}H_{14}O_3$: C, 65.93; H, 7.74%. Found: C, 65.80; H, 7.79%. Methoxyl: Calcd.; 17.04. Found; 16.95%.

Preparation of the α -Methyl Ether, $C_6H_5CHOCH_3.CHOH.CH_2OH$

1:1 Phenyl vinyl carbinol was prepared according to the method described by Moureu and Gallagher (18) using phenyl magnesium bromide and acrolein. 1:1 Phenyl vinyl carbinol (32.9 gm.) dissolved in 25 cc. of dry ether was methylated with 71 gm. of methyl iodide and 60 gm. of silver oxide. A colorless limpid oil (25.9 gm.) was obtained; b.p., 77.5–78.5° C./14–15 mm.; yield, 71.3%. Analysis:— Calcd. for $C_{10}H_{12}O$: C, 81.08; H, 8.10%. Found: C, 80.55; H, 8.05%.

Oxidation of the above methyl ether with dilute potassium permanganate gave 3.2 gm. of colorless crystals; m.p., 68° C.; yield, 26.0%. Analysis:— Calcd. for $C_{10}H_{14}O_3$: C, 65.93; H, 7.74%. Found: C, 66.18; H, 7.74%.

RING PARTITION OCCURRING DURING THE CONDENSATION OF
 α -PHENYL GLYCEROL WITH ACETONE

Condensation of α -Phenyl Glycerol with Acetone

α -Phenyl glycerol (21.7 gm.) was mixed with 140 gm. of pure dry acetone (20 times the theoretical amount) and 25 gm. of anhydrous copper sulphate added. The mixture was stirred, with a mechanical stirrer, for five days at 60° C., although it had become homogeneous, except for a layer of copper sulphate, after ten hours. After filtering, the acetone was removed under reduced pressure and 23.6 gm. of a slightly viscous oil obtained; b.p., 150–157° C./11 mm.; yield, 87.4%. Analysis:— Calcd. for $C_{12}H_{16}O_3$: C, 69.23; H, 7.69%. Found: C, 69.28, 69.32; H, 7.70, 7.75%.

The oil was then fractionally distilled in an effort to separate partially the two possible isomers, the second fraction being arbitrarily collected at about the middle of the boiling point range: (a) 13.4 gm: b.p., 151–154° C./11 mm.; (b) 9.0 gm.; b.p., 154–157° C./11 mm.

Methylation of the Isopropylidene α -Phenyl Glycerols

The above fractions of isopropylidene α -phenyl glycerol were methylated separately using silver oxide and methyl iodide; fraction (a) was treated with 37.2 gm. of methyl iodide and 30 gm. of silver oxide (approximately four times the theoretical quantity); fraction (b) with 26.0 gm. of methyl iodide and 21.6 gm. of silver oxide. The reaction was carried out in the manner described above for the methylation of cinnamyl alcohol, except that the mixture was stirred for two days after the last addition of silver oxide. On distillation, 8.5 gm. of oil was obtained from fraction (a); b.p., 115–117° C./5–6 mm.; $n_D^{20} = 1.4980$; and 7.0 gm. of oil from fraction (b): b.p., 111–114° C./5–6 mm.; $n_D^{20} = 1.4958$. Analysis:— Fraction (a): Calcd. for $C_{13}H_{18}O_3$: C, 70.27; H, 8.10%. Found C, 69.82, 69.81; H, 8.00, 8.00%. Mol. wt.: calcd., 222, Found (in $C_2H_5Br_2$), 238. Fraction (b): Calcd. for $C_{13}H_{18}O_3$: C, 70.27; H, 8.10%. Found: C, 69.96; H, 8.05%.

Hydrolysis of the Isopropylidene α -Phenyl Glycerol Methyl Ethers

Each of the above acetone methyl ethers was hydrolyzed by refluxing for six hours with 50 cc. of 70% methyl alcohol containing a drop of concentrated hydrochloric acid. The acid was neutralized with silver carbonate, the solutions filtered and concentrated. The solutions were then fractionally distilled with the following results:

The product from fraction (a) yielded 3.3 gm. of a pale yellow rather viscous oil; b.p., 135–140° C./4 mm. On seeding this oil with a few crystals of the α -methyl ether of phenyl glycerol and cooling to -12° C., 1.5 gm. of crystals was obtained which on recrystallization from benzene melted at 68–69° C. (Melting-point of the α -methyl ether of phenyl glycerol, 68° C.)

The product from fraction (b) yielded 3.0 gm. of a pale yellow oil; b.p., 126–128° C./3–4 mm. On seeding this oil with a few crystals of the γ -methyl ether of phenyl glycerol and cooling to -12° C., 1.0 gm. of white crystals was obtained; m.p., 54° C. (Melting-point of the γ -methyl ether of phenyl glycerol, 54° C.)

Condensation of the α -Methyl Ether of Phenyl Glycerol with Acetone

The α -methyl ether of phenyl glycerol (6.4 gm., m.p. 68° C.) was condensed with 70 gm. of dry acetone in the presence of 15 gm. of anhydrous copper sulphate as described above. The amount of isopropylidene α -phenyl glycerol methyl ether obtained was 3.0 gm.; b.p., 117–120° C./7 mm.; $n_D^{20} = 1.4979$. On hydrolysis 2.3 gm. of oil was obtained (b.p., 138–140° C./4 mm.) which crystallized in the condenser. Recrystallization gave a melting point of 68° C., corresponding with that of the α -methyl ether originally taken.

Condensation of the γ -Methyl Ether of α -Phenyl Glycerol with Acetone

On condensing 2.6 gm. of the γ -methyl ether of phenyl glycerol (m.p., 54° C.) with 35 gm. of acetone, 2.2 gm. of the γ -methyl ether of isopropylidene α -phenyl glycerol (b.p., 115–117° C./7–8 mm.; $n_D^{20} = 1.4958$) was obtained. Hydrolysis gave 1.5 gm. of oil (b.p., 130–132° C./4 mm.) which immediately solidified and on recrystallization melted at 54° C. that is, the same melting point as the γ -ether originally taken.

*Condensation of the α -Methyl Ether of Phenyl Glycerol with *p*-Nitrobenzaldehyde*

This condensation was carried out according to the method used for the preparation of the *p*-nitrobenzylidene glycerols (8). The α -methyl ether of phenyl glycerol (8.0 gm.) was condensed with 6.4 gm. of *p*-nitrobenzaldehyde, using one drop of 40% sulphuric acid as catalyst. An oil (12.3 gm.) was obtained which immediately solidified; yield, 92.0%. On recrystallization from a mixture of ethyl acetate and petroleum ether, very fine white crystals were obtained which softened at 58° C. and melted completely at 76° C. Analysis: Calcd. for $C_{17}H_{17}NO_5$: C, 64.76; H, 5.39%. Found: C, 64.70; H, 5.45%. On fractional crystallization two crops of crystals (*a*) rather large flat hexagonal crystals (m.p., 79–80° C.), and (*b*) rosettes of very fine, long white needles (m.p., 57–58° C.) were obtained. These two isomeric acetal methyl ethers have practically the same solubilities; they are easily soluble in cold benzene, ether and ethyl acetate, hot ligroin (b.p., 80–90° C.) but insoluble in water.

*Condensation of the γ -Methyl Ether of Phenyl Glycerol with *p*-Nitrobenzaldehyde*

The γ -methyl ether (7.6 gm.) was condensed with 6.0 gm. of *p*-nitrobenzaldehyde in the same manner. A viscous, dark red oil was obtained (11.2 gm.; yield, 90%) but, in spite of all efforts, the oil could not be induced to crystallize. An attempt to distil the oil resulted in decomposition at 250° C./0.024 mm. The oil was soluble in benzene, ether, and ethyl acetate and insoluble in alcohol and water. Analysis: Calcd. for $C_{17}H_{17}NO_5$: C, 64.76; H, 5.39%. Found: C, 64.67; H, 5.50%.

*Condensation of the α -Phenyl Glycerol with *p*-Nitrobenzaldehyde*

α -Phenyl glycerol (23 gm.) was condensed with 20 gm. of *p*-nitrobenzaldehyde at 120° C. (8). The dark red oil obtained was dissolved in benzene, washed with saturated sodium bisulphite solution, followed by dilute sodium bicarbonate and water, and dried over potassium carbonate. On cooling to –12° C., 12 gm. of yellowish needles and 22.6 gm. of viscous oil were obtained (total yield, 86.9%). The crystals were readily soluble in ether, acetone, warm benzene and alcohol but insoluble in ligroin and water. Analysis of crystals:— Calcd. for $C_{18}H_{18}NO_5$: C, 63.78; H, 4.98%. Found: C, 63.86; H, 5.06%.

The viscous oil has practically the same solubilities in organic solvents as the crystalline 1:3-*p*-nitrobenzylidene phenyl glycerol. Analysis of oil:— Calcd. for $C_{18}H_{18}NO_5$: C, 63.78; H, 4.98%. Found: C, 63.09; H, 5.19%.

PROOF OF THE STRUCTURE OF THE CRYSTALLINE 1:3-*p*-NITROBENZYLIDENE
1-PHENYL GLYCEROL.

*Methylation of 1:3-*p*-Nitrobenzylidene 1-Phenyl Glycerol.*

The crystalline 1:3-*p*-nitrobenzylidene α -phenyl glycerol (20 gm., m.p., 120–122° C.) was methylated using 40 gm. of methyl iodide and 30 gm. of silver oxide. Crystalline 1:3-*p*-nitrobenzylidene α -phenyl glycerol-2-methyl ether (10.4 gm.) was obtained (yield, 49.7%). The crystals had an indefinite melting point, 90–97° C. Analysis:— Calcd. for $C_{17}H_{17}NO_5$: C, 64.76; H, 5.39%.

Found: C, 64.62; H, 5.40%. On fractional recrystallization from benzene and petroleum ether two types of crystals were obtained: (a) a first crop of large white monoclinic crystals (m.p., 96° C.) soluble in cold benzene, ethyl acetate, ether, acetone and warm ligroin but insoluble in water; and (b) a second crop of small yellowish nodules (m.p., 113–115° C.), slightly more soluble in organic solvents than the previous isomer.

Acid Hydrolysis of the 1:3-p-Nitrobenzylidene Phenyl Glycerol-2-Methyl Ether

Ethyl alcohol (118 cc., 60%) containing 2 cc. of concentrated hydrochloric acid was added to 9.6 gm. of the above acetal methyl ether (m.p. 90–97° C.) and refluxed for eight hours. While warm, the solution was perfectly clear but on cooling to room temperature a considerable amount of solid separated out, m.p. 113–118° C., showing that this acetal is not so easily hydrolyzed under acid conditions as the analogous 1:3-*p*-nitrobenzylidene glycerol-2-methyl ether (8). Another cc. of hydrochloric acid was added and the mixture refluxed for a further eight hours. The solution was then neutralized by shaking with lead carbonate, filtered and evaporated to one quarter of its volume. On fractional distillation, 4.0 gm. of a pale yellow oil was obtained boiling at 153–156° C./5–6 mm. (yield, 74.5%). Analysis:— Calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74%. Found: C, 66.07; H, 7.80%.

When kept at 0° C. for three weeks, crystallization of the oil slowly took place, and after a further two weeks 3.5 gm. of white needle-like crystals of the β -methyl ether was obtained; m.p., 44° C. (The α -methyl ether of α -phenyl glycerol melts at 68° C. and the γ -methyl ether at 54° C.).

*Condensation of the β -Methyl Ether of Phenyl Glycerol with *p*-Nitrobenzaldehyde*

The above β -methyl ether (2 gm.) was condensed with 1.5 gm. of *p*-nitrobenzaldehyde. 1:3-*p*-Nitrobenzylidene phenyl glycerol-2-methyl ether (2.8 gm.) was obtained; m.p. 91–97° C.; yield, 82.4%.

Benzoylation of 1:3-p-Nitrobenzylidene Phenyl Glycerol

Crystalline 1:3-*p*-nitrobenzylidene phenyl glycerol (4 gm.) was dissolved in 6 gm. of dry pyridine and treated with 4 gm. of benzoyl chloride. The reaction flask was kept in cold water during the addition of the benzoyl chloride since a considerable amount of heat was generated in the reaction. The mixture, after standing overnight at room temperature, was poured on to cracked ice which caused the separation of a heavy oil; wt., 4.9 gm.; yield, 91.04%. From this, colorless crystals of the acetal benzoate were obtained; m.p., 141–143° C. The crystals were soluble in ethyl acetate and benzene, slightly soluble in ether and insoluble in water. Analysis:— Calcd. for $C_{23}H_{19}NO_6$: C, 68.15; H, 4.69%. Found: C, 68.00; H, 4.74%.

ATTEMPTED SEPARATION OF THE ISOMERIC FIVE-MEMBERED
p-NITROBENZYLIDENE α -PHENYL GLYCEROLS

Methylation of the Mixture of Five-membered Isomers

Sixteen grams of the dark red oil, remaining after separating the crystalline six-membered acetal as completely as possible, was methylated with methyl

iodide (50 gm.) and silver iodide (33 gm.). The oily product obtained was seeded with a few crystals of the 1-methoxy-2:3-*p*-nitrobenzylidene-1-phenyl glycerol which had been prepared previously as an identification compound. In spite of all efforts no crystalline product could be isolated from this oil (A).

Hydrolysis of the Methylated Mixture of Five-membered Isomers.

As no crystals could be obtained from this mixture, the oil was subjected to acid hydrolysis in order to isolate, if possible, the two isomeric methyl ethers of α -phenyl glycerol. A portion (11.4 gm.) of the product was dissolved in a small amount of benzene, and refluxed with constant stirring for eight hours with 120 cc. of 50% methyl alcohol containing 2 cc. of concentrated hydrochloric acid. The mixture did not become homogeneous at any time during this treatment, and on cooling to room temperature, 7.2 gm. of oil separated out. The alcoholic layer was decanted off and the residual liquid again hydrolyzed with 100 cc. of 50% alcohol containing 2 cc. of concentrated hydrochloric acid. The combined alcoholic solutions were neutralized by shaking with lead carbonate and then concentrated under reduced pressure to about one-quarter of the original volume. On cooling to 0° C., 3.1 gm. of *p*-nitrobenzaldehyde separated out. On distilling the residue, 3.7 gm. of a liquid was obtained; b.p. 160-164° C. The product was divided into two portions and seeded with crystals of the α -methyl ether of α -phenyl glycerol (m.p., 68° C.) and the γ -methyl ether (m.p., 54° C.) respectively. After cooling to -2° C. for two weeks, a small amount of a crystalline substance with a melting point of 52-54° C. was obtained, but none melting at 68° C. Definite evidence is thus obtained of the presence of one of the five-membered isomers (IV) in the mixture.

Benzoylation of the Mixture of Five-membered Isomers

A portion (7.5 gm.) of the oily mixture was benzoylated in dry pyridine with 7.5 gm. of benzoyl chloride. A considerable amount of heat was generated and a precipitate of pyridine hydrochloride separated almost immediately. No crystals could be obtained from the viscous product, however. Analysis of crude oil:— Calcd. for $C_{23}H_{19}NO_6$: C, 68.15; H, 4.69%. Found: C, 67.95; H, 4.75%.

Negative results in so far as concerned the isolation of crystalline products were also obtained on acetylation with acetic anhydride in pyridine and on *m*-bromobenzoylation with *m*-bromobenzoyl bromide in pyridine.

Preparation of the Trityl Derivative of one of the Isomers in the Acetal Mixture

Trityl chloride (7.5 gm.) was mixed with 5.0 gm. of the viscous oil dissolved in 16 gm. of dry pyridine, and the mixture heated to 100° C. for three hours. On cooling to room temperature, crystals of pyridine hydrochloride separated out. The mixture was poured on to cracked ice when a very viscous, heavy oil and a white solid precipitated out. The latter was readily decanted from the oil, filtered and recrystallized from ether. White crystals (2.9 gm.) were obtained melting at 158-160° C. (melting point of triphenyl carbinol, 162° C.). The heavy viscous oil was taken up in ether and the ether solution washed

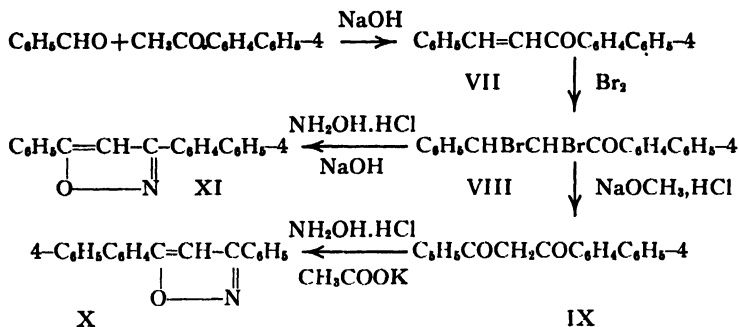
12 times with cold water to remove as much of the pyridine as possible. The solution was dried over sodium sulphate and the ether allowed to evaporate slowly in a vacuum desiccator over concentrated sulphuric acid. A small amount of crystalline triphenyl carbinol separated from the oily residue (B) but no other product.

Methylation of the residual oil (B)

A portion (7.0 gm.) of the residual oil (B) obtained above after removal of the triphenyl carbinol by filtration was methylated with 16 gm. of methyl iodide and 11 gm. of silver oxide. The methylated product was left as an oil, which could not be induced to crystallize on seeding an ether solution with crystals of the *p*-nitrobenzylidene derivative of the α -methyl ether of phenyl glycerol and cooling to -12° C. for six weeks.

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Experimental

The necessary *p*-phenylphenacyl bromide was prepared by the method of Drake and Bronitsky (4), using animal charcoal for decolorizing in both steps.* The acetate (V). A mixture of 10 gm. of potassium acetate, an equal weight of the bromide, and 150 cc. of 60% alcohol was acidified to litmus with acetic acid (this prevented the formation of colored by-products) and warmed on a steam bath for three hours. On cooling, the ester separated as white leaflets in yields of 90-95%; m.p., 111° C.

The carbinol (IV). A mixture of 5 gm. of the acetate in 50 cc. of alcohol containing 1 cc. of concentrated sulphuric acid was refluxed for three hours, the flask cooled in a freezing mixture, and the crystals filtered and recrystallized from alcohol, using animal charcoal for decolorizing. It formed white leaflets, m.p. 123-28° C.; yield: 3.8 gm. or 91%. A sharp melting point could not be obtained, in spite of repeated recrystallizations from different solvents. It is insoluble in water but readily soluble in organic solvents. Analysis:— Calcd. for $\text{C}_{14}\text{H}_{12}\text{O}_2$: C, 79.4%; H, 5.7%. Found: C, 79.1%; H, 5.8%. A great many unsuccessful attempts were made to accomplish the hydrolysis by methods recorded in the literature for similar esters; only the above gave a good result.

p-Phenylphenacyl carbinol formed a phenylurethane with phenylisocyanate, re-formed the acetate with acetyl chloride, and gave a 2,4-dinitrophenylhydrazone. The carbinol (0.5 gm.) and 0.7 gm. of phenyl isocyanate in a small test tube were heated a few minutes in a dish of hot water; the mixture solidified on cooling. It was removed and recrystallized from alcohol; m.p. 186-7° C. Analysis:— Calcd. for $\text{C}_{14}\text{H}_{17}\text{O}_3\text{N}$: N, 4.2%. Found: N, 4.1%. The acetate was made by allowing a solution of the carbinol in acetyl chloride to evaporate to dryness and recrystallizing from alcohol. It melted at 111° C., and a mixed melting point with the acetate made from the bromide was likewise 111° C. The 2,4-dinitrophenylhydrazone separated at once when 1 gm. of carbinol in 10 cc. of warm alcohol was added to 1 gm. of 2,4-dinitrophenylhydrazine in 2 cc. of concentrated sulphuric acid and 15 cc. of alcohol. It was recrystallized from a mixture of three parts of ethyl acetate and one part alcohol, and formed scarlet needles, m.p. 222-23° C. with decomposition. It is slightly soluble in methyl and ethyl alcohols, chloroform and benzene. Analysis:— Calcd. for $\text{C}_{20}\text{H}_{16}\text{O}_6\text{N}_4$: N, 14.3%. Found: N, 14.1%.

*The writers are indebted to the Dow Chemical Co., Midland, Michigan, for the diphenyl used in this work, and take this opportunity to express their appreciation of this assistance.

Benzal p-phenylacetophenone (VII) (3, p. 196). To a mixture of 2 gm. of the ketone and 1.2 gm. of benzaldehyde in 60 cc. of hot alcohol was added 1 cc. of 50% potassium hydroxide solution, and the whole heated in boiling water under a reflux condenser for a half-hour. On cooling, the unsaturated ketone crystallized; m.p. 156° C. The yield was 2.7 gm. or 93%; 60 gm. of diphenyl gave 100 gm. of unsaturated ketone in two steps, an over-all yield of 90%. Bergmann and Wolff (2) recently prepared this same ketone in a yield of 63–65% by the action of cinnamoyl chloride on diphenyl.

Benzal p-phenylacetophenone dibromide (VIII). The unsaturated ketone (2.8 gm., 0.01 mole) in 30 cc. of warm carbon disulphide was cooled in running water and a solution of 1.6 gm. of bromine in 20 cc. of the same solvent added with stirring. After standing for ten minutes at room temperature with occasional shaking the solid was filtered and washed with aqueous sodium bisulphite; the yield was 3 gm. and a further 0.5 gm. of solid was obtained from the filtrate (a total yield of 75%). On recrystallization from benzene it formed a white powder, m.p. 189° C. It is decomposed on boiling with alcohols. Analysis:— Calcd. for $C_{21}H_{18}OBr_2$: Br, 36.0%. Found: Br, 36.1%.

Benzoyl p-phenylbenzoylmethane (IX). This was made by the published method for dibenzoylmethane (1, p. 199) in a yield of 80%; it formed fine light yellow prisms, m.p. 112° C., and gave a deep red color with ferric chloride. Analysis:— Calcd. for $C_{21}H_{18}O_2$: C, 84.0%; H, 5.3%. Found: C, 83.7%; H, 5.2%. The copper derivative was made by shaking an ethereal solution of the diketone with a saturated aqueous solution of copper acetate. It separated as an olive-green powder, insoluble in alcohol, ether, and benzene. After washing thoroughly with water and alcohol it melted at 290° C. with decomposition. Analysis:— Calcd. for $C_{42}H_{30}O_4Cu$; Cu, 9.6%. Found: Cu, 9.4%.

3-Xenyl-5-phenylisoxazol (X). The potassium chloride was filtered from a mixture of 2 gm. of potassium acetate, 1 gm. of hydroxylamine hydrochloride, and 15 cc. of glacial acetic acid, 8 gm. of the diketone added to the filtrate, and the whole refluxed for two hours. Water was then added and the precipitated solid filtered and recrystallized from benzene. It separated in pearly leaflets, m.p. 182–83° C.; yield; 0.8 gm., 70%. Analysis:— Calcd. for $C_{21}H_{18}ON$: N, 4.7%. Found: N, 4.5%.

3-Phenyl-5-xenylisoxazol (XI). A mixture of the above dibromide, 1 gm. of hydroxylamine hydrochloride, 50 cc. of alcohol, and 4 cc. of 40% aqueous sodium hydroxide was refluxed for a half hour. The white solid that separated on cooling was filtered and recrystallized from benzene. The yields were only 0.6–0.7 gm. (40–50%). It also formed pearly leaflets, m.p. 195° C. Analysis:— Calcd. for $C_{21}H_{18}ON$: N, 4.7%. Found: N, 4.9%.

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EXPERIMENTS ON THE PYROLYSIS OF GASEOUS PARAFFINS, WITH SPECIAL REFERENCE TO THE PRODUCTION OF ETHYLENE¹

BY ADRIEN CAMBRON²

Abstract

On passing natural gas, containing 4.8% ethane, through an electrically heated tungsten spiral, the formation of liquid products was observed at 1050° C.

On passing the same gas over an electrically heated 4-mm. carbon rod enclosed in an uninsulated silica tube 2.0 cm. wide, 7.7% of the gas was converted to liquid and solid hydrocarbons at a rod temperature of 1050-1100° C. No liquids or solids except carbon were formed when the silica tube was insulated.

By replacing the 2.0-cm. silica tube by a water-cooled Pyrex tube, 3.2 cm. wide, no liquid or solid hydrocarbons were obtained, but 17% of the gas was converted to gaseous unsaturated hydrocarbons at 993° C. On passing ethane through the 3.2-cm. water-cooled reaction tube at rod temperature of 989° C., 50.5% of the entering gas was converted to ethylene, and 90.0% of the ethane decomposed did so according to the equation $C_2H_6 \longrightarrow C_2H_4 + H_2$.

The dehydrogenation of ethane was found to be greatly accelerated by the presence of reduced copper in the tube, the metal being used in the form of a cylindrical wire gauze and placed around the carbon rod. Copper bronze coated with molybdic oxide was also found to be active under the same conditions.

Introduction

A concise but very comprehensive review of the literature on the pyrolysis of paraffin hydrocarbons was published by Lomax *et al.* (5). This review contains a chronological index of the literature on the subject, covering the work published between 1809 and 1915. The work done on the same subject is reviewed in greater detail in a paper by G. Egloff, R. E. Schaad and C. S. Lowry, Jr. (1), and which covers the literature up to 1930.

The procedure generally followed for studying the pyrolysis of gaseous hydrocarbons consists in passing the gases through externally heated tubes. The present work was undertaken with the object of studying the pyrolysis of natural gas and of ethane in a different type of apparatus. Instead of using externally heated tubes, in which the temperature of the reaction space is governed by the temperature of the walls of the tube, internally heated reaction tubes were used, the source of heat being an electrically heated carbon rod placed axially in the middle of the tube. This arrangement allowed the average temperature of the reaction space surrounding the rod to be varied between wide limits while the surface temperature of the latter was kept constant.

Experiments with Natural Gas

The sample of gas used was from Turner Valley, Alberta. It was taken after passage through a separator, and may be considered as representative of

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the dry gas from this field. An analysis carried out in a Podbielniak fractionating apparatus showed it to have the following composition :-

Methane.....	89.7% (by volume)
Ethane.....	4.8%
Propane.....	3.4%
Butanes.....	1.5%
iso.....	0.83%
normal.....	0.67%
Pentanes and higher hydrocarbons.....	0.6%*

The gas was run through a Burrell apparatus in this laboratory, and found to be free from carbon dioxide, oxygen and nitrogen.

PYROLYSIS OVER ELECTRICALLY HEATED TUNGSTEN SPIRAL

Peters and Meyer (8) have described a method for converting methane into acetylene in good yield by passing the gas under reduced pressure through an electrically heated spiral of tungsten at 3000° C. At a pressure of 50 mm. and a contact period of 0.0001 sec. they obtained a product containing 15.4% acetylene; 66.5% of the entering methane having been converted to acetylene on one passage. A serious disadvantage of this procedure would appear to be the very short life of the spiral, due to the formation of tungsten carbide.

In the present author's first experiments with natural gas, an electrically heated tungsten spiral was used as the source of heat and the reaction vessel was similar to that employed by Peters and Meyer. It was found that by maintaining the spiral at lower temperatures than the latter employed, liquid hydrocarbons could be secured. Even, however, at the optimum temperature, *viz.*, about 1050° C., the yield of such hydrocarbons was only 2-3% of the gas introduced. Experiments were carried out both at atmospheric and at 50 mm. pressure. The spirals used were 0.6 cm. outside diameter by 4.0 cm. long, and were made of 1.0-mm. tungsten wire. The use of tungsten spirals as source of heat was abandoned because carbide formation, which appears to be quite rapid even at 1050° C., limits the life of the spirals to a few hours, and it did not appear promising to repeat the above experiments at a lower temperature, since Hilpert and Ornstein (3), on heating finely divided tungsten in the presence of methane, observed the formation of carbide at temperatures as low as 800° C.

PYROLYSIS OVER ELECTRICALLY HEATED CARBON ROD

Experiments in Quartz Tubes

In all the subsequent experiments described herein, the source of heat was an electrically heated carbon rod (made from electrode carbon), and the reaction vessel, a tube in which the rod was placed axially. In the first series of experiments, the results of which are given in Table I a rod 4 mm. in diameter and various sizes of silica tubes were used. The rod required about 25 amperes to bring its temperature up to 1050-1100° C. Some difficulty was experienced

* The author is much indebted to Mr. P. V. Rosewarne, Mines Branch, Ottawa, for making this analysis.

in determining the temperature of the rod in the quartz tube experiments; an approximate reading was obtained by exposing about 0.5 cm. of the rod beyond the exit end of the tube and observing the rod with an optical pyrometer. Such readings were somewhat low but permitted duplication of experiments at the same temperature.

TABLE I

INFLUENCE OF DIAMETER OF QUARTZ REACTION TUBE ON YIELD OF LIQUIDS

No.	Length of rod, cm.	Diameter of silica tube, cm.	Contact period, sec.	Yield of liquids and solid hydrocarbons,* %
34	7.6	0.68	0.075	3.7
35	14.6	0.88	0.35	6.75
36	14.6	0.88	0.80	6.95
37	14.6	2.0	10.1	7.7
38	14.6	2.0 (insulated)	10.1	Trace

NOTE:— Rod temperature: 1050-1100° C.

* The yield of liquids and solids is given in per cent of the weight of the gas put through.

The quartz reaction tubes used in the first four experiments were not insulated, and the temperature of the walls of the tube was consequently lower, especially in the case of the wider tube, than the rod temperature. The results of the last experiment, in which the silica tube was insulated, and in which, consequently, the walls of the tube were at a higher temperature than in the preceding experiment, are noteworthy, since there was formed practically no liquid or solid hydrocarbons, but an abundant deposit of carbon.

Experiments in Water-cooled Pyrex Reaction Tube

In consequence of the observation just mentioned, the silica tube was replaced by a water jacketed tube of Pyrex glass. It was thought that in such a reaction vessel any liquid products formed would condense on the walls and thus escape decomposition. Instead, however, of obtaining high yields of liquid hydrocarbons with this arrangement, the yields of such products were negligible but, on the other hand, the yields of acetylene and ethylene were remarkably high.

The results obtained at a rod temperature of 1050° C., for instance, with a water-cooled tube, are in sharp contrast with results obtained by previous experimenters with tubes heated externally at about the same temperature. Passage through the latter type of tube leads to the conversion of a considerable proportion of methane and other gaseous paraffins into aromatic hydrocarbons. The temperature gradient in the two types of reaction vessels is of course entirely different. It would appear that in a tube externally heated at 1050°-1080° C., there is a sufficiently extensive zone at the lower temperatures which favors the production of aromatics from ethylene and acetylene (cf. Hague and

Wheeler (2)) to allow polymerization of a considerable proportion of the unsaturated gases formed by the cracking of the paraffin hydrocarbons on the hot walls. In a tube of the type employed in the present experiments, however, with its sharper temperature gradient between the rod and the walls, such a zone is small, and the initial unsaturated products formed after thermal activation of the paraffin molecules by contact with the hot rod diffuse out into the relatively cold regions surrounding the rod before polymerization can take place.

Influence of Gas Rate on Formation of Unsaturated Hydrocarbons

Table II gives the results of the experiments at a rod temperature of 1050° C., in a water-cooled Pyrex tube of 3.2 cm. diameter. The carbon rod, 0.4 cm. in diameter and 14.0 cm. long, was as before disposed axially in the tube.

These experiments were designed to ascertain the influence of the rate of flow of the entering gas on the concentration of unsaturated gases in the pyrolyzed gas. The figures given for ethylene include any propylene which may have been present.

TABLE II
INFLUENCE OF RATE OF FLOW, AT ROD TEMPERATURE OF 1050° C.,
ON YIELD OF UNSATURATED HYDROCARBONS

No.	Flow, l./hr.	Expansion, %	Analysis of product			Yield (based on weight of entering gas)	
			C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₂ %	C ₂ H ₄ %
48	6.5	27	2.1	5.35	24.9	3.75	9.16
49	7.9	30	2.2	5.4	23.8	4.02	10.7
50	11.4	30	2.6	5.6	23.4	4.75	11.1
59	14.2	28	2.7	6.0	19.0	4.85	11.7
51	15.8	30	2.3	6.3	20.3	4.20	12.4

Although the maximum concentration of ethylene in the exit gas did not appear to have been reached, this series of experiments was not carried further, because of the fact that the rapid deposition of carbon at the temperature in question (1050° C.) made it difficult to keep the temperature constant, owing to gradual reduction in the resistance of the rod.

It is of interest to contrast these results obtained at 1050° C. in a water-cooled internally heated tube, with those obtained by Stanley and Nash (11) at 1150° C. in an externally heated tube. In a 5-mm. silica tube and with a heating period of 0.6 seconds, the latter found that 11% of the entering methane was converted to light oil and tar and 8.8% to acetylene and ethylene, a total conversion to higher hydrocarbons of 19.8%. In the present experiments almost as high a yield of higher hydrocarbons was obtained, but these were composed exclusively of acetylene and ethylene, practically no polymerization of these primary products to liquid hydrocarbons having taken place.

Influence of Temperature on Formation of Unsaturated Hydrocarbons

In Table III are given the results of experiments made to determine the influence of the rod temperature on the yields of unsaturated gases. It will be seen that increase in temperature produces a fall in the yield of ethylene and a rise in that of acetylene.

TABLE III
INFLUENCE OF TEMPERATURE ON YIELD OF UNSATURATED HYDROCARBONS

No.	Temp., ° C.	Flow, l./hr.	Expansion, %	Analysis of product			Yield (based on weight of entering gas)	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₂ %	C ₂ H ₄ %
67	993	14.2	14.0	1.90	8.3	11.0	3.04	14.3
63	1020	14.0	22.5	1.81	7.42	13.9	3.11	13.8
62	1040	14.0	26.5	1.64	7.20	16.2	2.92	13.7
57	1050	14.2	25.0	2.25	6.70	17.05	3.96	12.7
45	1060	14.7	36.0	2.40	3.6	25.8	4.58	7.4
42	1200	15.1	56.0	3.5	2.3	55.1	7.65	5.4

The low ratio of the amount of hydrogen to the amount of ethylene and acetylene found in the pyrolyzed gas at 993° C. would seem to show that, at this temperature, the formation of these unsaturated hydrocarbons is probably due in greater part to the decomposition of the ethane and propane in the natural gas than to that of the methane.

Effect of Volume of Reaction Space on Yield of Unsaturated Hydrocarbons

A series of experiments was carried out with water-cooled reaction tubes of different sizes, viz., 1.92, 3.20 and 3.87 cm. diameter. The heating rod was in each case 14 cm. long and 0.4 cm. in diameter. The results, which are summarized in Table IV, show that within the limits tried the size of the reaction tube is without marked effect on the course of the reaction at an approximately constant rate of gas flow.

TABLE IV
INFLUENCE OF VOLUME OF REACTION SPACE ON REACTION

No.	Temp. ° C.	Reaction space, cc.	Flow, l./hr.	Space velocity, l./l./sec.	Expan- sion, %	Analysis of product			Yield (based on weight of enter- ing gas)	
						C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₂ %	C ₂ H ₄ %
54	1050	40.5	16.2	0.111	25	2.5	6.7	25.0	4.4	12.6
53	1055	121	15.8	0.036	29	2.8	6.0	22.7	5.1	12.7
59	1047	165	14.2	0.024	28	2.7	6.0	19.0	4.9	11.6

The reaction space as given in the above table is the volume of the space between the carbon rod and the walls of the reaction tube.

The fact that the reaction appears to be independent of the size of the reaction tube can probably be best explained as follows. Since contact between the gas and the rod is brought about by convection, the lower space velocity in the case of the wider tube permits recirculation of the gas over the rod to a greater extent than in the narrower tube. Actually, in order to favor recirculation of the gas over the rod, the tube is set at an angle of about 15°, the gas entering the tube at the upper end. By placing a few lumps of aluminum chloride inside the tube, and mixing water vapor with the gas by bubbling it through water, the mist produced inside the tube permitted observation of the flow of the gas in the tube. It was observed that the entering gas, on coming in contact with the upper end of the rod, flows back along the upper part of the tube, mixing with the fresh entering gas as it cools, the same process being repeated after each contact, so that the gas flows over the rod several times before reaching the outlet at the lower end of the tube. It is obvious that recirculation will be more rapid in a narrow tube and, consequently, although the gas remains in the tube a shorter time the actual contact period must be about the same in both cases.

Effect of Copper Gauze on Yield of Unsaturated Hydrocarbons

It was found that by placing a cylinder of copper gauze between the hot rod and the cold walls of the reaction tube, and thus cutting down the loss of heat through radiation, the current required to keep the rod at a given temperature could be decreased. Table V gives the results of some experiments made in this connection. The gauze used, 40-mesh, was in the form of a cylindrical spiral, 2.6 cm. in diameter and 15 cm. long, which completely surrounded the rod.

TABLE V
EFFECT OF COPPER GAUZE ON CURRENT CONSUMPTION AND YIELD
OF UNSATURATED HYDROCARBONS

No.	Temp., °C.	Copper gauze	Current watts	Flow, l./hr.	Expan- sion, %	Analysis of product			Yield (based on weight of enter- ing gas)	
						C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₂ %	C ₂ H ₄ %
63	1020	Without	450	14.0	22.5	1.81	7.42	13.94	3.1	13.8
80	1020	With	346	14.4	13.2	1.35	7.69	15.60	0.8	13.1
62	1040	Without	604	14.0	24.0	1.64	7.20	16.24	3.1	13.5
95	1040	With	407	35.9	14.0	2.39	7.07	21.76	3.8	12.2

It will be observed that the current consumption is greatly reduced by the use of the copper gauze.

There is another point of interest in the above results. There is a marked reduction in the expansion when the copper spiral was used, although the amount of hydrogen formed under these conditions is greater than in the absence of the spiral. The lower expansion in the presence of the metal spiral

was due to the formation and condensation of liquid products on the walls of the tube. The formation of these liquid products can be explained by the presence, between the copper spiral and the rod, of a reaction zone at a sufficiently high temperature for the polymerization of the unsaturated hydrocarbons which are formed as primary products. Probably the conditions inside the spiral were quite similar to the conditions inside of the uninsulated silica tube as used in experiment No. 37 (Table I). The condensation of liquid products on the walls of the tube was actually observed in the course of the two experiments with copper gauze; unfortunately, the form of apparatus used at the time did not permit their quantitative estimation.

Experiments with Ethane

Ethane was subjected to pyrolysis in the same form of apparatus as that used in the last experiments with natural gas. The jacketed reaction tube was 3.2 cm. in diameter and the carbon rod 0.4 cm. in diameter and 14.0 cm. long.

The gas used had the composition:—ethane, 95.01; methane, 3.78; nitrogen, 1.21% by volume.

Effect of Rod Temperature on Concentration of Ethylene in Pyrolyzed Gas

The work of Pease and Durgan (7) shows that the dehydrogenation of ethane to ethylene by thermal treatment is an equilibrium reaction,



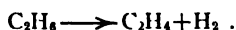
At 650° C., for instance, the dissociation of ethane into ethylene and hydrogen was found to be 27.5%. As the reaction from left to right is endothermic, the extent of dissociation increases with temperature. This dissociation, however, is a slow reaction even at temperatures as high as 700° C., and, further, side reactions always displace the equilibrium, so that the equilibrium concentration of ethylene is never attained in stream experiments, that is, when ethane is passed through a hot tube.

It is interesting to contrast the internally heated reaction tube herein described with externally heated tubes used by earlier experimenters. Hague and Wheeler (2) passed ethane through an externally heated quartz tube 2.2 cm. in diameter and 70 cm. long. The temperature in the middle of the tube was determined by a thermocouple inside a narrow quartz tube placed axially within the reaction tube. At a temperature of 950° C., as registered by the thermocouple in the middle of the tube, and a gas flow of 4 litres per hour, 13.9% of the ethane put through decomposed to carbon and hydrogen, and the exit gas contained only 3.8% ethylene and 1.4% unchanged ethane, along with 52.6% hydrogen and 40.8% methane. In the form of reaction tube used in the present experiments, even when the temperature in the middle of the tube, *i.e.*, the temperature of the carbon rod, was as high as 989° C., no carbon was formed, and the exit gas was very much higher in ethylene and unchanged ethane than in Hague and Wheeler's experiments, the concentration of ethylene being 28.83, that of ethane, 28.65%.

TABLE VI
EFFECT OF TEMPERATURE ON ETHYLENE CONCENTRATION

No.	Temp., ° C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through %	Ethane cracked %
70	969	13 13	37 8	0 69	27 38	28 26	34 4	7 68	39 6	79 5
78	989	12 6	56 8	1 45	28 83	32 71	28 65	6 03	47 2	90 0
68	1000	13 5	57 0	1 60	28 2	34 9	21 6	11 0	46 7	72 6
69	1007	13 3	57 5	2 0	28 0	35 5	21 1	11 6	46 5	71 2

From the above results the optimum temperature appears to be about 990° C. at a gas rate of 12.6 l. per hour. Under these conditions half of the ethane put through is converted to unsaturated hydrocarbons, while 90% of the ethane which decomposed did so according to the equation



As one would expect, the amount of acetylene and methane formed increase with increase in temperature. The fraction reported as ethylene actually contains about 8% of propylene, but probably less than 1% of butylene and butadiene.

Effect of Rate of Flow on Concentration of Ethylene in Product

On determining the effect of the rate of flow of ethane through the water-cooled reaction tube on the concentration of ethylene in the pyrolyzed gas, the results recorded in Table VII were obtained.

TABLE VII
EFFECT OF RATE OF FLOW ON ETHYLENE CONCENTRATION

No.	Temp., ° C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through	Ethane cracked
78	989	12 6	56 8	1 45	28 83	32 78	28 65	6 03	47 2	90 0
86	993	28 92	34 0	0 76	25 47	26 23	37 73	8 09	37 1	74 5
108	985	45 5	20 2	0 39	17 23	16 34	57 59	5 89	21 2	73 0

The concentration of ethylene in the exit gas decreases quite rapidly with increase in gas flow and this would indicate that recirculation of the gas over the rod by convection is not as rapid as believed or that the rod itself possesses little or no activity as a dehydrogenation catalyst. Subsequent experiments will show that the latter appears to be the case.

Effect of Reduced Copper on Dehydrogenation of Ethane

As already pointed out, the dehydrogenation of ethane to ethylene is a true equilibrium. It was consequently thought that with a suitable catalyst higher rates of flow of ethane could be used without lowering the concentration of ethylene in the product at a given temperature.

In the first experiments on the use of a catalyst a cylindrical copper gauze, 2.15 cm. in diameter and 15 cm. long, made of 35-mesh gauze, was placed in the reaction tube around the carbon rod. Before using the gauze it was oxidized in oxygen at 250° C., and placed in the tube in this form. The film of oxide was rapidly reduced by the hydrogen formed in the reaction. The results obtained with this arrangement are recorded in Table VIII.

TABLE VIII
EFFECT OF REDUCED COPPER ON CONCENTRATION OF ETHYLENE IN PRODUCT

No.	Temp., ° C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on:	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through	Ethane cracked
81	985	7.62	58.3	1.40	30.37	38.55	12.05	13.97	50.7	59.5
82	985	15.5	57.2	1.39	30.17	36.35	18.52	11.40	49.8	67.0
83	993	20.2	41.7	1.34	31.35	35.52	21.60	8.58	46.7	64.0

In the above experiments, the distance between the copper gauze and the rod was 9 mm., the temperature of the gauze being about 450° C. The increase in the concentration of ethylene and the fact that the concentration of ethylene does not decrease with increase in gas rate (cf. Table VII), indicate that reduced copper under the above conditions undoubtedly catalyses the dehydrogenation of ethane. Unfortunately the copper was rapidly attacked, a loose deposit containing copper and carbon forming on the gauze, so that the circulation of the gas through the gauze was stopped in a short time.

Effect of Metallic and Reduced Copper Bronze Gauze on Reaction

The difficulties experienced with the pure copper spiral were avoided to a certain extent by making the contact gauze, shaped as before in the form of a cylinder, of 40-mesh copper bronze (50-50 CuSn), and by using a cylinder of 2.6 cm. instead of 2.15 cm. in diameter, the temperature of the gauze under these conditions being 398° C., as measured by a thermocouple.

No gauze was used in the first of the experiments recorded in Table IX, the results being included for comparison with the second experiment, in which the 2.6 cm. cylindrical gauze was used without any surface treatment. In the third experiment, the gauze was superficially oxidized before placing it in the reaction tube.

TABLE IX
ACTION OF METALLIC AND REDUCED CuSn GAUZE ON REACTION

No.	Contact	Temp., °C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on:	
					C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through %	Ethane cracked %
86	None	993	28.92	34	0.76	25.47	26.23	37.73	8.09	36.0	72.6
87	Metallic CuSn	993	29.0	36	0.88	25.82	26.72	38.78	6.98	37.0	78.5
85	Reduced CuSn	993	28.9	47.5	0.99	29.93	33.57	24.12	9.82	46.5	72.2

It will be observed that the analyses of the products from the two first experiments are very closely the same, showing that the rate of dehydrogenation of ethane has not been affected by the metallic CuSn gauze; the presence of the latter, however, by cutting down radiation losses, reduces by 20% the current required to keep the rod at 993° C. The increased rate of dehydrogenation of ethane in the presence of the reduced CuSn gauze is shown by the higher ethylene concentration in the product, and also by the higher conversion of ethane to ethylene.

Effect of Rate of Flow on Ethylene Yield in the Presence of CuSn Gauze

The experiments recorded in Table X were all carried out in the presence of a 2.6-cm. reduced CuSn gauze, the same gauze being used throughout.

TABLE X
EFFECT OF RATE OF FLOW ON ETHYLENE YIELD IN THE PRESENCE OF CuSn GAUZE

No.	Temp., °C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on:	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through %	Ethane decom- posed %
84	993	19.74	53.3	1.16	31.26	36.53	19.02	9.86	50.5	71.5
85	993	28.9	47.5	0.99	29.93	33.57	24.12	9.82	46.5	72.2
88	993	32.6	44.0	0.90	29.70	32.34	29.60	6.00	45.1	81.8
98	990	33.8	44.0	0.99	27.66	30.95	28.84	9.68	41.8	75.6
99	985	34.0	40.5	0.84	27.07	29.59	32.34	8.86	40.1	79.0
97	985	40.0	41.5	0.76	26.30	28.26	33.64	9.74	39.2	78.6

The ethylene concentration in the product dropped abnormally after the third experiment, and the probable reason for this was later found to be the contamination of the rod by copper from the gauze. It was observed that the CuSn gauze, although less readily attacked than pure copper, became corroded

on prolonged use, this corrosion resulting in the formation of a loose deposit on the gauze. Some of this deposit apparently fell on the rod, causing the formation on the latter of a film of dull carbon. It can be assumed that copper at 989° C. would strongly catalyze the decomposition of ethane to carbon and hydrogen, since this metal has an appreciable effect on the decomposition of the more stable methane to its elements at 910° C., according to W. E. Slater (10). Subsequent experiments will also show that alteration of the rod surface, such as produced by the deposition of a film of dull carbon, can have a marked effect on the course of the reaction.

Influence of Rod Surface on Reaction

When carbon rods, such as have been used in the course of the present work, are electrically heated to 1030-1050° C. in natural gas, they become covered with a thin film of silvery-looking carbon. If similar rods are heated in ethane at 985° C., the original dull surface of the rod also becomes covered by a smooth film of carbon, but the latter is much darker in color and less shiny in appearance than when the rod is heated in natural gas at a higher temperature.

The experiments recorded in Table XI were carried out to determine the difference in apparent activity of carbon rods before and after causing the film of lustrous carbon to form on their surface by heating them in natural gas. (These experiments were carried out in the presence of copper-tin gauze.)

TABLE XI
EFFECT OF ROD SURFACE ON REACTION

	Current, watts	Flow, l./hr.	Expansion, %
Untreated rod	313	49.2	32.0
Same rod, heated in natural gas at 1027° C.	308	49.0	41.0
Untreated rod	311	48.5	30.5
Same rod, heated in natural gas at 1040° C.	305	48.7	40.7
Same rod, heated in natural gas at 1040° C.	305	65.5	30.5

Two different rods were used in the above experiments. The expansion observed when ethane is subjected to pyrolysis is a fairly accurate index of the ethylene concentration in the gas produced, provided of course that, as was the case, no appreciable amounts of carbon or liquid products are formed. It will be observed that the expansion with the first untreated rod was 32%, and 30.5 with the second, and this corresponds to a concentration of ethylene in the pyrolyzed gas of about 25.5% (cf. experiment No. 112, Table XII). After heating the same carbon rods in natural gas at 1030-1040° C. in order to produce a lustrous film on the surface, the expansion observed after passing ethane over the rod was 41.0% with the first rod and 40.5% with the second. This corresponds to a concentration of ethylene in the pyrolyzed gas of 28.5%. (In fact

the product obtained at 308 watts with the first rod analyzed 28.47% ethylene). Still more striking evidence as to the marked effect of the nature of the rod surface on the reaction was obtained in the fifth experiment, when the rate of flow of the ethane was increased to 65.5 litres per hour, and the same expansion observed as at 48.5 litres per hour with the untreated rod. Since a 30.5% expansion corresponds to an ethylene concentration in the exit gas of 25.5%, and consequently to a 33% conversion of entering gas to ethylene, the presence of the shiny film of carbon on the rod has apparently effected an increase in the rate of production of ethylene from 16 to 22 litres per hour in the two experiments referred to.

It is doubtful whether the apparent greater activity of the rod covered with a film of shiny, silvery-looking carbon is to be ascribed to a specific catalytic action of this form of carbon on the dehydrogenation of ethane, for one would expect this dense and compact form of carbon to be quite inactive. In fact, Seidenschnur and Jäppelt (9) observed that the film of carbon formed on coke by passing methane over this material at 800° C. started to reduce CO₂ at 610° C.; if, however, a film of dense grey carbon was formed on the coke by passing methane over it at 1030° C., the coke then had to be heated to 690° C. before reduction of CO₂ could be observed.

It seems that the greater apparent effectiveness in the present experiments of the silvery as compared with the dull carbon surface is due to the fact that for a constant current, owing to the lower emissivity of the former surface, the actual temperature of the silvery rod is higher than that of the dull one. (The formation of the thin film of hard lustrous carbon causes no appreciable difference in the resistance of the rod). The higher rod temperature naturally brings about a more rapid rate of dehydrogenation of the ethane, the inertness of the surface preventing decomposition of ethane to carbon and hydrogen.

The observed temperatures of two 4-mm. rods one treated with natural gas at 1040° C., the other untreated and consequently with a dull black surface, were found to be 985° C. and 978° C. respectively at 310 watts, the temperature being determined by means of a Leeds & Northrup optical pyrometer. The observed temperature in the case of the rod with a dull surface probably represents fairly accurately the true temperature of the rod; in the case of the silvery rod, however, which has an emissivity of about 0.5 (as determined approximately by measuring the light reflection of the surface), as compared to an emissivity of about 0.9 for the dull rod, the optical pyrometer reading does not represent the actual rod temperature. From curves published in Tech. Paper 170, U.S. Bureau of Standards, the positive correction which must be applied to a pyrometer reading of 985° C. in the case of a surface having an emissivity of 0.5 is 50° C. Actually, however, since probably about 50% of the heat generated in the rod is carried away by thermal conduction or absorbed in the process of activation of the gas, the above correction should be divided by two, so that the true temperature corresponding to a reading of 985° C. is probably about 1010° C. in the case of the shiny rod.

Effect of CuSn Gauze Coated with MoO₃ on Reaction

As already mentioned, reduced copper or copper-tin gauze has been found to have a definite catalytic action on the dehydrogenation of ethane. A serious disadvantage of using copper in this manner is that the metal is quite rapidly corroded, even when the copper-tin alloy is used. It was found that treatment of the CuSn gauze with molybdic oxide (MoO₃), which itself is known to act as a dehydrogenation catalyst, appeared completely to obviate corrosion of the metal without appreciably affecting the activity of the contact. After 30 hr. use a contact gauze treated in this manner showed no visible signs of corrosion. It was also found that, due to the protective action of the oxide, the gauze could be placed closer to the heated rod. In the following experiments, except the first one, two cylindrical contacts were used (these were made of CuSn gauze treated with MoO₃), one of 2.25 cm., the other of 2.65 cm. diameter, the purpose of the latter being to prevent channeling of the gas between the inner cylinder and the walls of the tube. The temperature of the inside surface of the inner contact was measured with a thermocouple and found to be 460° C. Some of the results obtained with this arrangement are recorded in Table XII.

TABLE XII

EFFECT ON ETHYLENE CONCENTRATION OF DOUBLE GAUZE COATED WITH MOLYBDIC OXIDE

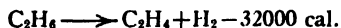
No.	Temp., ° C.	Flow, l./hr.	Expan- sion, %	Analysis of product					Yield of ethylene (in %) based on:	
				C ₂ H ₂ %	C ₂ H ₄ %	H ₂ %	C ₂ H ₆ %	CH ₄ %	Ethane put through	Ethane cracked
108	985	45.5	17.1	0.39	17.23	16.34	57.59	5.89	21.2	73
109	985	51.6	37.0	0.55	27.82	29.25	32.76	8.21	40.1	76.1
110	985	48.0	34.8	0.56	26.89	27.91	34.75	9.48	40.9	80.5
111	982	49.0	41.0	1.08	28.47	31.43	32.58	5.88	42.2	81.5
112	985	65.5	30.5	0.57	25.34	26.13	39.55	6.75	34.9	76.2

No gauze was used in the first of the above experiments, the results being included for purposes of comparison. The current consumption in the above experiments ranged between 300 and 319 watts.

Results previously recorded (cf. Table IX) have shown that the percentage of ethane converted to ethylene by a single passage through the water-cooled tube at a gas flow of 29 litres per hour, is increased from 35.8 to 46.5% by using reduced CuSn gauze as contact. The results recorded in the above table show that by coating the gauze with molybdic oxide and placing it closer to the rod, the influence of the gauze on the rate of dehydrogenation of ethane becomes still more marked, since the percentage of ethane converted to ethylene on one passage at 45.5 litres per hour was 21.2% in the absence of a contact, but rose to over 40% in the presence of the gauze, and this at a slightly higher gas rate.

Conclusion

According to Nernst (6, p. 780), if a glowing metallic filament is placed in a gas capable of dissociating, there would probably be only very slight recombination of the dissociation products during the short time required for these to diffuse from the surface of the filament out into the comparatively cold layers a short distance from it. Furthermore, if dissociation was rapid enough, there would be equilibrium at the surface of the filament, and hence the cold gas some distance from the filament would have the same composition as if the whole of the gas were uniformly heated to the temperature of the filament. Langmuir (4) confirmed Nernst's views by showing that on passing water vapor or carbon dioxide through a tube containing a glowing platinum wire, the amounts of the dissociation products obtained are those which correspond to the dissociation equilibrium at the temperature of the wire. It would consequently be expected that by passing ethane over a glowing carbon rod in a water-cooled reaction tube, as was done in the present work, the amounts of ethylene and hydrogen obtained would correspond to the dissociation equilibrium of ethane at the temperature of the rod. Actually it has been found that the concentration of ethylene approached the equilibrium concentration corresponding to the rod temperatures used and was in excess of that corresponding to the average temperature of the space. It did not actually attain the former concentration even at low gas rates, due to limiting factors which were absent in the reactions studied by Langmuir, such as the lack of catalytic activity on the part of the rod and the displacement of equilibrium by side reactions. The results obtained show, however, that the formation of unsaturated hydrocarbons under the conditions of the above experiments is most probably a surface reaction. In support of this view may be mentioned the fact that in several experiments the thermal efficiency of the water-cooled reaction tube, that is, the fraction of the energy supplied as electrical energy which is actually used up in the reaction:



is about 12%. It is highly improbable that under the conditions of these experiments, this amount of energy could be absorbed by the gas in the form of radiant energy, instead of by actual contact of the gas with the rod.

On the other hand, some of the results obtained seem to show that the polymerization or decomposition of the initial unsaturated products to liquid products or to carbon and hydrogen are space reactions, and take place when the space surrounding the rod is kept at a sufficiently high temperature. This can be readily understood when it is considered that both are exothermic reactions, and require only a sufficiently high temperature to initiate them.

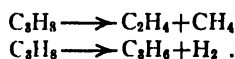
Trial Experiments with Propane and Butane

Experiments have been carried out with samples of propane and butane, using the same apparatus and temperatures as in the last experiments recorded in Table XII, the object being to determine the behavior of these paraffins in the water-cooled reaction tube. Results are recorded in Table XIII.

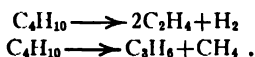
TABLE XIII
YIELD OF OLEFINS FROM PROPANE AND BUTANE

No.	Gas used	Temp., ° C.	Flow, l./hr.	Expan- sion, %	Analysis of product						
					C ₂ H ₂ %	C ₂ H ₄ %	C ₃ H ₆ %	H ₂ %	CH ₄ %	C ₃ H ₈ %	C ₄ H ₁₀ %
113	Propane	970	51.8	35.8	0.86	16.85	8.43	12.78	22.64	37.85	—
114	Butane	962	48.3	47.5	0.84	21.30	9.67	8.89	21.95	—	35.10

The results from the propane experiment show that 70.7% of the propane which decomposed on one passage through the tube did so according to the following equations:



Similarly with butane, 62.1% of the gas which decomposed on one passage did so as follows:



(The propane and butane used have not been analyzed, and in making these calculations are assumed to be 100% pure.)

The above results show that when propane and butane are pyrolyzed by contact with a glowing carbon rod, the main products consist of olefins. It will be observed that in the case of propane the predominating reaction is demethanation to ethylene, while some propylene is also formed by straight dehydrogenation. With butane, the predominating reaction is the opening of the 2:4 carbon-to-carbon bond, giving rise to two moles of ethylene and one mole of hydrogen, while a third of the olefins produced consist of propylene formed by demethanation. The amounts of butylene and butadiene formed in both of the above experiments were very small compared to the other olefins produced.

Further experiments are being carried out with propane and butane in the same form of apparatus, particularly to determine whether the conversion to the corresponding olefine can be increased by lowering the temperature and using a suitable catalyst.

Acknowledgments

The author wishes to acknowledge the interest of Dr. G. S. Whitby, Director, Division of Chemistry, National Research Laboratories, in the work. He is also greatly indebted to Mr. C. H. Bayley for carrying out the gas analyses.

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STRUCTURAL VISCOSITY IN SOME LYOPHILIC SOLS

I. THE FLOCCULATION OF GELATIN AND CASEIN BY AGAR¹

BY WILFRED GALLAY²

Abstract

The relative viscosities of mixtures of gelatin and agar sols were measured at varying pressures using the overflow viscosimeter. All the sols showed wide deviations from Poiseuille's law, with regions of structure and turbulence at low and high pressures, respectively. A minimum viscosity was found for a intermediate mixture, showing a dehydrating action of the agar on the gelatin. This effect was masked in a similar series using casein and agar, due to the much greater difference in relative viscosity of the two sols. A marked structural turbulence was found in the region of structure in many cases. The relation between velocity of flow and pressure is expressed by a parabolic equation.

The most characteristic property of a lyophilic sol is its comparatively high relative viscosity, the latter increasing sharply with higher concentrations of solutes. In the case of a true solution the velocity of flow is usually found to be directly proportional to the pressure, according to Poiseuille's law

$$V = K \frac{P}{\eta},$$

where V is the velocity of flow, P the pressure, η the viscosity, and K a constant.

Owing to turbulence, deviations from the law occur at high pressures, where the relation becomes an exponential one. Ostwald and collaborators (5, 6) have further generalized this relation by taking into consideration the results obtained in the measurement of the viscosity of lyophilic sols at low pressures. Further aberrations from Poiseuille's law have been found under these conditions, the velocity of flow decreasing enormously at low rates of shear. This must obviously be ascribed to a structure effect in the sol, the effect being nullified at higher pressures. With a sufficiently high concentration of solute, only a small proportion of the solvent is free, the rest being bound by the highly solvated micelles. At low rates of shear, the tendency towards an incipient gel formation is sufficiently great to show evidence of a structure in the sol. As the pressure increases, owing to the comparatively ready deformability of the solvated solute, the resistance to flow decreases with consequent nearer approach to a linear relation between pressure and velocity of flow.

With further increased rate of shear, it is found that the relative viscosity in many cases increases. This has been ascribed to a turbulent effect in the sol. Since this effect appears in the case of lyophilic sols at a much lower pressure than in the cases of liquids and true solutions, it would seem that the ordinary mass turbulence does not here obtain, but that the effect is probably one of loss of kinetic energy by the solute particles in directions other than that of flow.

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The generalized relation of Ostwald is shown diagrammatically in Fig. 1, for pressure and velocity of flow, and in Fig. 2 for pressure and viscosity. Lyophobic sols in many cases show all three regions. In some instances, as shown below, the region where Poiseuille's law is obeyed disappears entirely, the structural region passing over gradually into that of turbulence.

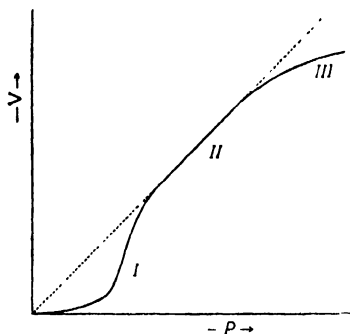


FIG. 1. Diagrammatic representation of velocity of flow-pressure relation. I, Structure; II, Poiseuille's law; III, turbulence.

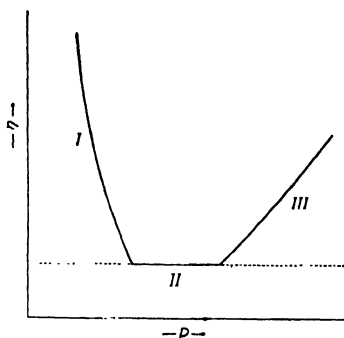


FIG. 2. Diagrammatic representation of viscosity-pressure relation. I, Structure; II, Poiseuille's law; III, turbulence.

These measurements can be made readily by the use of the ordinary capillary viscosimeter with an added arrangement for the maintenance of a constant pressure during the flow of the liquid. Particularly convenient, however, is the apparatus of Ostwald and Auerbach (4) as shown in Fig. 3.

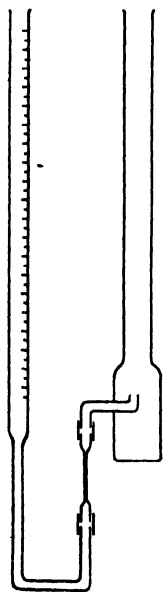


FIG. 3. Overflow viscosimeter.

In this apparatus the surface tension and kinetic energy effects obtaining at the outlet of the ordinary consistometer are obviated and the readings taken of the velocity of flow themselves serve as measurements of the hydrostatic head of pressure on the sol.

Flocculation of a colloiddally dispersed system can be brought about by one or both of two general methods, *viz.*,

1. By the lowering of the electrokinetic potential through the addition of a suitable electrolyte or of an oppositely charged colloid.

2. By desolvation usually brought about by the addition of a liquid lyophobe to the dispersed phase and miscible with the solvent.

Tiebackx (9, 10) found that, within narrow ranges of hydrogen ion concentration, flocculation was obtained upon mixing sols of gelatin and gum arabic. The precipitate partook somewhat of the nature of casein or globulin in being peptized by dilute alkalis and somewhat stronger acids, with a zone of flocculation at intermediate pH. He ascribed this to a mutual discharge between the carbohydrate and protein particles. Ostwald and Hertel (7, 8)

extended this to reactions between various starches and gelatin and between agar and gelatin. The extent of flocculation depended to some extent upon the relative concentrations. The starches showed no charge whatever and the gelatin a weak positive charge by electrophoretic measurement, and Ostwald and Hertel point out that this, then, may hardly be regarded as a process of flocculation by mutual discharge. These authors tend rather to the view that here a desolvation occurs, the more strongly hydrated colloid withdrawing a part of the bound solvent from the less strongly hydrated colloid. In support of this view they found, in the case of gelatin and starch, that the coagulum consisted almost entirely of starch, the less strongly hydrated colloid.

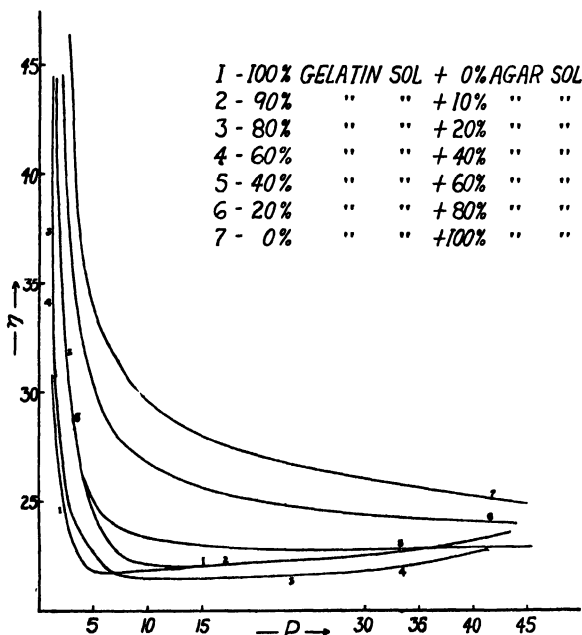


FIG. 4. Viscosity of gelatin-agar mixtures.

in the beginning of the structural region at about 10 cm. pressure (sol No. 2) instead of at about 5 cm. pressure (sol. No. 1). Poiseuille's law is not obeyed at any pressure, the two curves merging into one at about 13 cm. pressure, passing from the structural region to that of turbulence. The curves for sols Nos. 3 and 4 (20 and 40% of the agar sol) are identical. Here it will be noticed that the viscosity of the sol is actually appreciably lowered where Poiseuille's law is more nearly obeyed. In No. 5 the viscosity is much higher as is to be expected, and at pressures above 20 cm. the velocity of flow is very nearly proportional to the pressure. Nos. 6 and 7 show great increases in viscosity and show structure over the whole range of pressures measured. Instead of steadily increasing viscosity from No. 1 to No. 7, as would be expected from a merely additive effect, the lowest viscosity is shown by intermediate mixtures, owing to the dehydration of the gelatin by the agar.

The viscosity of a lyophilic sol increases with increase of solvent bound by the disperse phase and, other factors being equal, may be taken to be a relative measure of the degree of hydration of the colloid. The viscosities of mixtures of varying proportions of a 1% gelatin sol and a 0.2% agar sol were measured by means of the Ostwald-Auerbach viscosimeter. No macroscopic flocculation was evident other than some noticeable turbidity in the sols containing 20 and 40% of the agar sol. The viscosity-pressure relation for this series is shown in Fig. 4.

All of the sols show wide deviations from Poiseuille's law. The effect of the presence of 10% of the agar sol is noticed

The viscosities of mixtures of varying proportions of a 2% basic casein sol and a 0.25% agar sol were similarly measured. The results show a regular increase in viscosity with increasing concentration of the agar sol. It is very probable that the wide variation in relative viscosities between the two sols has had the effect of masking the dehydrating action of the agar upon the casein. The results are shown in Fig. 5.

Blair (1) has stated that there is no necessity of a parabolic equation for the expression of the relation between velocity of flow and pressure and has attempted to show in several examples that a linear relation will suffice. Ostwald (3), citing a long list of examples, has shown that the parabolic equation is necessary in general, the

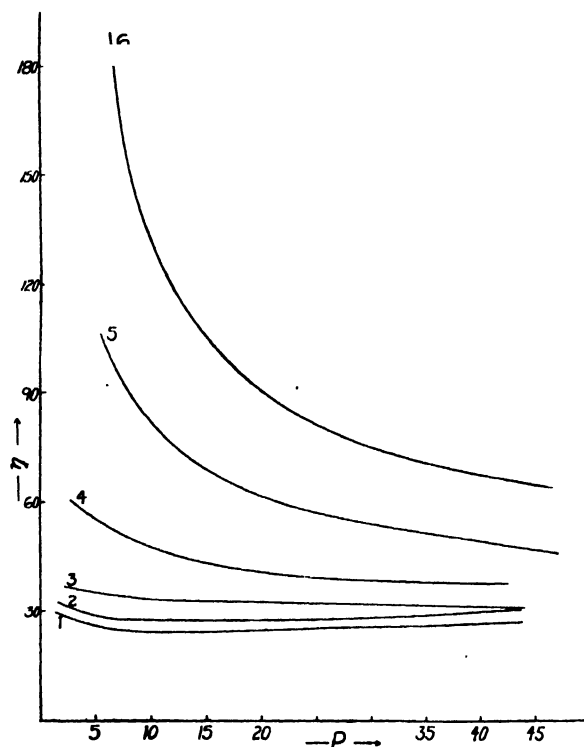


FIG. 5. Viscosity of casein-agar mixtures. Curve 1, sol C. Curve 2, 65 cc. C plus 10 cc. D. Curve 3, 50 cc. C plus 25 cc. D. Curve 4, 40 cc. C plus 35 cc. D. Curve 5, 15 cc. C plus 60 cc. D. Curve 6, sol D.

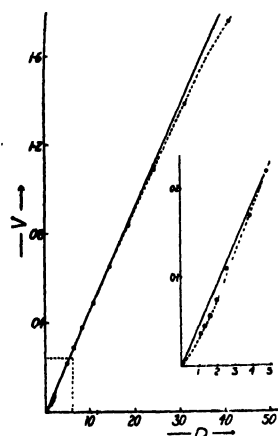


FIG. 6. Velocity of flow-pressure curve corresponding to Curve 1, Fig. 4.

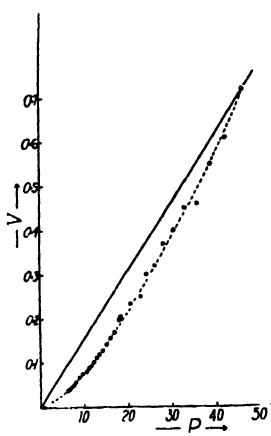


FIG. 7. Velocity of flow-pressure curve corresponding to Curve 6, Fig. 5.

cases chosen by Blair having been fortuitously close to the linear relation.

In Figs. 6 and 7 are shown two typical examples of the velocity of flow-pressure curves. In Fig. 6 (Curve 1, Fig. 4) the structural region (enlarged in the inset) is shown as a slight but quite regular deviation from the line and convex to the pressure axis, and the turbulent region at higher pressures concave to the pressure axis. In Fig. 7 (Curve 6, Fig. 5), an example is shown

where the condition is structural throughout. It may be concluded that the generalization diagrammatically represented in Fig. 1 is substantiated, and that the relation between velocity of flow and pressure cannot be represented by an equation of the first order.

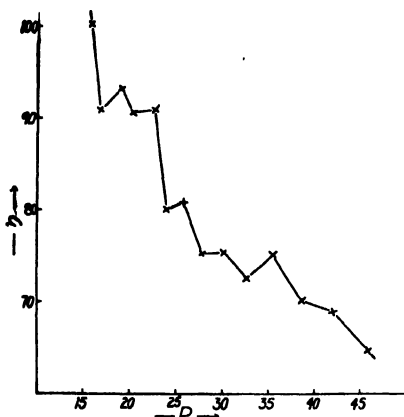


FIG. 8. Structural turbulence (first portion of Curve 6, Fig. 5.)

Fig. 8 reproduces a portion of Curve 6, Fig. 5, showing the actual experimental values obtained. Although the total effect is clearly one of structure, portions of the curve show actual rise in viscosity with increase in pressure. This is analogous to results obtained by Hatschek and Jane (2) with ammonium oleate sols, and Ostwald (5) has termed this effect one of structural turbulence as distinguished from the turbulence at higher pressures. It has been explained by Ostwald as being due to a tearing effect on the structure leading to turbulent motion in the sol. Most of the sols measured show

this typical irregularity in the structural region.

Experimental

Viscosity measurements were carried out with the overflow viscosimeter in a thermostat kept at 30°C. In the case of aqueous sols, the capillary was attached by means of rubber tubing, and for the benzene sols, by corks coated with a lacquer insoluble in benzene. With the aid of an assistant, readings were taken, at equal time intervals, of the height of liquid in the burette tube. From these were calculated the volume of flow during the time interval, the average velocity of flow for a definite pressure, and finally the relative viscosity at each pressure. In all cases readings were taken over short time intervals (10 or 20 sec.) and then means were taken of successive groups to give the average volumes of flow for 10 sec. during successive minutes.

In the tables below, excepting Table VII, only these condensed sets of figures are shown. In all cases P is the pressure in cm. of solution, V the velocity of flow in cc. per 10 sec. and $\eta = \frac{P}{V}$ is the relative viscosity. Table VII shows the total calculations involved.

The gelatin used was the Eastman's ash-free leaf product and the agar a purified fine white powder obtained from Schuchardt's. The basic casein sol was prepared from "casein puriss" of Schuchardt by the use of an excess of calcium oxide.

TABLE I
VISCOSITY OF SOL A

P	V	η
41.24	1.76	23.44
31.44	1.39	22.62
24.44	1.09	22.42
18.70	0.84	22.26
14.27	0.65	21.96
10.88	0.49	22.20
8.28	0.38	21.79
6.31	0.29	21.76
4.82	0.22	21.91
3.86	0.17	22.71
2.54	0.11	23.09
2.00	0.075	26.67
1.61	0.057	28.24
1.32	0.045	29.33
1.10	0.037	29.73

Note:—Sol. A, 0.5% gelatin;
Sol B, 0.2% agar.

TABLE II
VISCOSITY OF 90% OF
A PLUS 10% OF B

P	V	η
41.15	1.76	23.38
31.72	1.40	22.66
24.30	1.09	22.30
18.57	0.84	22.11
14.17	0.64	22.14
10.83	0.49	22.10
8.30	0.37	22.43
6.38	0.28	22.79
4.99	0.20	24.95
4.10	0.16	26.00
2.83	0.087	32.53
2.34	0.075	31.20
1.97	0.053	36.87
1.66	0.043	38.34

TABLE III
VISCOSITY OF 80% OF
A PLUS 20% OF B

P	V	η
41.08	1.81	22.69
31.49	1.42	21.89
23.95	1.11	21.58
18.14	0.84	21.60
13.74	0.64	21.47
10.38	0.49	21.18
7.85	0.37	21.22
5.98	0.27	22.14
4.57	0.20	22.86
3.67	0.163	23.71
2.46	0.093	26.45
2.00	0.068	29.41
1.62	0.053	30.57
1.36	0.038	35.79
1.16	0.03	38.67

TABLE IV
VISCOSITY OF 60% OF
A PLUS 40% OF B

P	V	η
41.08	1.80	22.82
31.51	1.44	21.88
23.91	1.10	21.74
18.10	0.84	21.54
13.70	0.64	21.41
10.39	0.48	21.65
7.91	0.38	20.82
6.01	0.27	22.26
4.61	0.20	23.05
3.70	0.15	24.18
2.52	0.10	25.71
2.01	0.072	27.92
1.63	0.052	31.35
1.39	0.040	34.75
1.19	0.027	43.89

TABLE V
VISCOSITY OF 40% OF
A PLUS 60% OF B

P	V	η
41.23	1.97	22.83
31.82	1.39	22.84
24.45	1.08	22.72
18.82	0.82	22.90
14.50	0.63	23.08
11.20	0.48	23.36
8.68	0.37	23.50
6.77	0.28	24.04
5.31	0.21	24.33
4.37	0.17	25.88
3.07	0.10	31.33
2.52	0.08	31.50
2.10	0.063	33.33
1.78	0.046	38.58
1.54	0.035	43.86

TABLE VI
VISCOSITY OF 20% OF
A PLUS 80% OF B

P	V	η
41.61	1.73	24.05
32.60	1.33	24.51
25.55	1.04	24.57
20.09	0.80	25.11
15.85	0.62	25.56
12.60	0.48	26.25
10.05	0.37	27.16
8.04	0.30	26.80
6.49	0.23	28.22
5.45	0.19	28.68
3.98	0.12	33.17
3.35	0.10	34.53
2.84	0.078	36.41
2.43	0.055	44.18
2.13	0.052	42.60

TABLE VII
 VISCOSITY OF SOL B

Time		P	P (average)	Δ	V	V (average)	η
min.	sec.						
0	10	48.0	41.80	2.00		1.66	25.18
	20	46.0		1.80	1.90		
	30	44.2		1.70	1.75		
	40	42.5		1.60	1.65		
	50	40.9		1.50	1.55		
1	0	39.4	33.12	1.60	1.55	1.27	26.08
	10	37.8		1.45	1.53		
	20	36.35		1.35	1.40		
	30	35.00		1.30	1.33		
	40	33.70		1.27	1.29		
2	0	32.43	26.32	1.23	1.25	1.01	26.06
	10	31.20		1.15	1.19		
	20	30.05		1.17	1.16		
	30	28.88		1.08	1.13		
	40	27.80		1.00	1.04		
3	0	26.80	20.99	1.05	1.03	0.78	26.91
	10	25.75		0.95	1.00		
	20	24.80		0.93	0.94		
	30	23.87		0.88	0.91		
	40	22.99		0.83	0.86		
4	0	22.16	16.91	0.86	0.85	0.60	28.18
	10	21.30		0.78	0.79		
	20	20.58		0.72	0.75		
	30	19.80		0.70	0.74		
	40	19.10		0.65	0.68		
5	0	18.45	13.68	0.65	0.65	0.48	28.50
	10	17.80		0.62	0.64		
	20	17.18		0.59	0.61		
	30	16.59		0.59	0.59		
	40	16.00		0.55	0.57		
6	0	15.45	11.15	0.57	0.56	0.38	29.34
	10	14.88		0.50	0.54		
	20	14.38		0.49	0.50		
	30	13.89		0.47	0.48		
	40	13.42		0.43	0.45		
6	0	12.99	11.15	0.45	0.44	0.38	29.34
	10	12.54		0.42	0.44		
	20	12.12		0.42	0.42		
	30	11.70		0.36	0.39		
	40	11.34		0.41	0.39		

TABLE VII—Continued

Time		P	P (average)	Δ	V	V (average)	η
min.	sec.						
	50	10.93			0.38		
7	0	10.59		0.34	0.35		
	10	10.24		0.35	0.35		
	20	9.90		0.34	0.34		
	30	9.57		0.33	0.33		
	40	9.25	9.12	0.32	0.32	0.31	29.42
	50	8.93		0.32	0.30		
8	0	8.66		0.27	0.28		
	10	8.38		0.28	0.28		
	20	8.11		0.27	0.27		
	30	7.85		0.26	0.26		
	40	7.60		0.25	0.23		
	50	7.40	7.50	0.20	0.23	0.25	30.00
9	0	7.14		0.26	0.25		
	10	6.90		0.24	0.24		
	20	6.67		0.23	0.22		
	30	6.46	6.38	0.21	0.19	0.195	32.72
	40	6.29		0.17	0.18		
	50	6.10		0.19	0.19		
	60	5.92		0.19			
10	30	5.40					
11	0	4.95	4.69	0.45	0.44	0.137	34.23
	30	4.52		0.43	0.38		
12	0	4.20	4.05	0.32	0.32	0.107	37.85
	30	3.89		0.31	0.32		
13	0	3.57	3.44	0.32	0.30	0.09	38.22
	30	3.30		0.27	0.24		
14	0	3.10	3.00	0.20	0.20	0.067	44.77
	30	2.90		0.20	0.20		
15	0	2.71	2.63	0.19	0.18	0.057	46.14
	30	2.55		0.16	0.16		
16	0	2.40		0.15			

TABLE VIII

Visc. SOL C

P	V	η
42.31	1.61	26.28
33.83	1.26	26.85
26.98	1.04	25.94
21.38	0.85	25.15
16.84	0.67	25.13
13.30	0.54	24.63
10.43	0.43	24.26
8.19	0.33	24.82
6.44	0.26	24.77
5.29	0.20	26.45
3.60	0.13	27.70
2.90	0.11	27.10
2.36	0.082	28.78
1.90	0.067	28.36

TABLE IX

Visc. 65 cc. C PLUS 10 cc. D

P	V	η
42.56	1.40	30.40
34.59	1.20	28.83
28.04	1.00	28.04
22.64	0.81	28.10
18.29	0.66	27.71
14.72	0.53	27.71
11.87	0.42	28.26
9.59	0.35	27.40
7.74	0.27	28.67
6.48	0.24	27.00
4.57	0.17	27.36
3.73	0.13	29.37
3.05	0.10	29.90
2.52	0.078	32.31

TABLE X

Visc. 50 cc. C PLUS 25 cc. D

P	V	η
43.14	1.41	30.6
35.65	1.12	31.8
29.53	0.92	32.1
24.55	0.75	32.7
20.43	0.63	32.7
17.05	0.52	32.8
14.23	0.43	33.1
11.90	0.36	33.1
9.95	0.30	33.2
8.57	0.26	33.6
7.00	0.21	34.1
5.86	0.165	35.8
4.97	0.142	35.0
4.20	0.12	35.0
3.55	0.10	36.6

NOTE:- Sol C, 2% casein;
Sol D, 0.25% agar

TABLE XI

Visc. 40 cc. C PLUS
35 cc. D.

P	V	η
38.99	1.04	37.49
33.28	0.87	38.25
28.49	0.74	38.76
24.46	0.61	40.00
21.08	0.52	40.54
18.24	0.44	41.93
15.85	0.37	42.84
13.82	0.31	44.58
12.10	0.27	44.82
9.06	0.185	49.00
6.34	0.122	51.97
4.57	0.081	56.42

TABLE XII

Visc. 15 cc. C PLUS
60 cc. D

P	V	η
45.44	0.97	47.1
40.26	0.79	50.9
35.84	0.69	51.8
32.02	0.59	54.4
28.68	0.53	54.6
25.79	0.45	58.0
23.28	0.40	58.2
20.33	0.33	62.2
18.48	0.29	63.7
14.40	0.21	69.9
11.31	0.14	79.1
9.07	0.11	84.0
7.44	0.08	94.2
6.20	0.06	101.6

TABLE XIII

VISCOSITY OF SOL D

P	V	η	P	V	η
45.93	0.71	64.69	12.73	0.113	112.6
42.04	0.61	68.92	12.11	0.102	120.2
38.61	0.55	70.20	11.54	0.093	124.1
35.46	0.46	77.09	11.00	0.088	125.0
32.67	0.45	72.60	10.49	0.080	131.1
30.16	0.40	75.40	10.05	0.073	137.8
27.86	0.37	75.30	9.60	0.075	128.0
25.85	0.32	80.78	9.19	0.067	137.2
24.01	0.30	80.03	8.80	0.067	131.3
22.72	0.25	90.88	8.43	0.067	147.9
20.38	0.24	90.58	8.10	0.057	142.1
19.10	0.21	93.17	7.79	0.057	147.0
16.80	0.17	90.86	7.47	0.053	155.6
15.85	0.155	102.2	7.21	0.048	171.7
14.97	0.142	105.4	6.94	0.042	154.2
14.17	0.128	110.7	6.68	0.045	147.0
13.43	0.120	111.8	6.47	0.040	184.8
				0.035	

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STRUCTURAL VISCOSITY IN SOME LYOPHILIC SOLS

II. RUBBER SOLS¹

BY WILFRED GALLAY²

Abstract

The relative viscosities of sols of natural and purified rubber in benzene were measured at varying pressures using the overflow viscosimeter. Deviations from Poiseuille's law to varying extents were found in all cases, with regions of structure and turbulence at various pressures. The limiting concentrations above which structure is evident and Poiseuille's law is not obeyed were found to be in agreement with those calculated by Staudinger, on the basis of his theory of the relation between the viscosity and molecular weight of polymeric molecules. Exposure to ultra-violet light was found to decrease considerably the viscosity of a sol of purified rubber.

The equation of Einstein expressing a linear relation between specific viscosity and the volume occupied by the solute, based partly upon the assumption of non-deformable spheres, has been found to hold for certain sols, e.g., mastic. It has been found to fail utterly, however, in the case of such sols as gelatin and rubber. The relative viscosity of such sols increases enormously with increase in concentration, and on the basis of Einstein's equation, attempts at calculations of the volume occupied by the solute often yield figures many times greater than the actual volume of solvent used.

Staudinger (2) has found the following empirical relation to hold for various polymeric series,

$$\frac{\eta_{sp}}{c} = K.M,$$

where η_{sp} is the specific viscosity, c the concentration, M the molecular weight, and K , a constant for any one series in the same solvent.

Assuming that the extent of solvation is small, possibly only an adsorption film, Staudinger considers that the effective volume of a long-chain molecule is that of a short cylinder encompassing the rotatory movements of the molecule. Upon this assumption, the effective volume occupied by the molecule must be proportional to the square of the molecular weight or chain length. In a dilute sol containing solute molecules of less than a certain size, the volume of solvent available is sufficient for free movement of the particles. In a sol of the same concentration containing very long molecules, however, the number of molecules decreases inversely as the chain length, but the effective volume increases as the square of the chain length, and a condition is attained where the volume of solvent available no longer suffices for free movement of the particles. Staudinger has calculated the effective volumes of various high polymers and the limiting concentrations above which normal solution changes to "gel solution".

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It is evident that this condition of mutual hindrance in the movement of solute particles must be synonymous with the structural region, discussed elsewhere (1), where there is no conformity with Poiseuille's law. The extent of solvation is considered by most workers to be very great. However, the interaction between the bound solvent in the outer parts of the spheres of attraction of two molecules, from the point of view of viscosity, is unknown.

Under these conditions also, the magnitude of the force binding the liquid at some distance from the hydrocarbon chain is very uncertain.

In order to test the theory of Staudinger from the point of view of the critical concentrations above which normal solution is no longer possible, the viscosity of a number of rubber sols was investigated by means of the overflow viscosimeter.

Fig. 1 shows the viscosity-pressure curves of sols of various concentrations of acetone-extracted smoked sheet. Neglecting for the moment the initial rise in viscosity, it is seen

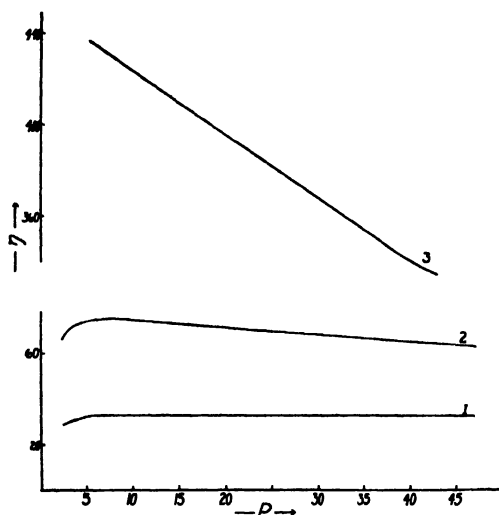


FIG. 1. Viscosity of sols of natural rubber. Concentrations;—1, 0.19%; 2, 0.38%; 3, 0.96%

that the relative viscosity is independent of the pressure with a concentration of 0.19%, but the structural effect is very marked at a concentration of 0.38%. Staudinger (3) gives 0.2% as the limiting concentration in the case of raw Hevea rubber.

Fig. 2 shows the viscosity-pressure curves for sols of various concentrations of purified rubber. The rubber was purified by the diffusion method (described in the experimental section) after acetone extraction, and showed no nitrogen present by the sodium fusion test. The sol of 0.42% concentration shows no structure, and the sol of 1.06% concentration shows marked structure. The limiting concentration given by Staudinger (3) for a rubber sol purified according to the method of Pummerer is 0.61%. This figure is for the more readily soluble portion of the rubber (the so-called sol-rubber) as purified according to Pummerer. In the present instance, only the most readily diffusible portion of the rubber was used, representing only about 5% of the total weight of rubber employed. In order of magnitude, at least, the results of these viscosity measurements are in agreement with the theory of Staudinger.

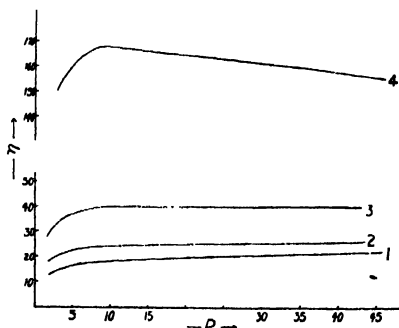


FIG. 2. Viscosity of sols of purified rubber. Concentrations;—1, 0.11%; 2, 0.21%; 3, 0.42%; 4, 1.06%

Fig. 3 shows the effect of exposure to ultra-violet light (6 hr.) of sols of natural and the purified smoked sheet. The sols were contained in loosely stoppered quartz flasks with air above the sol. It is well known that the removal of nitrogenous matter from the rubber results in a large decrease in viscosity, but it is evident that the structure is due to the hydrocarbon. The effect of exposure to ultra-violet light also causes a decrease in viscosity, not only in the sol of natural rubber but also in that of the purified hydrocarbon, and therefore it may be concluded that the effect is chiefly, if not entirely, upon the hydrocarbon itself.

In nearly all of these viscosity-pressure curves for rubber sols, it will be noticed that the first part shows an increased viscosity with rise in pressure. On the basis of long unbranched hydrocarbon chains, this initial rise is very difficult of explanation. On the other hand, assuming the presence of branched chains, there is a possibility of initially increasing interference with the tendency towards orientation of the molecules.

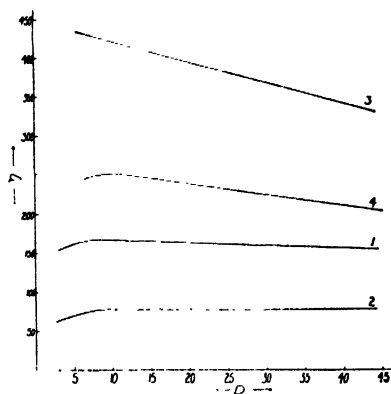


FIG. 3. Effect of ultra-violet light on viscosity of rubber sols. Purified rubber, concentration 1.06%: 1, before exposure; 2, after exposure. Natural rubber, concentration 0.96%: 3, before exposure; 4, after exposure.

Experimental

Natural smoked sheet was extracted with dry acetone for ten days at about 30° C., dried *in vacuo* and sols were prepared in pure benzene at room temperature. The viscosities were measured with the overflow viscosimeter immersed in a thermostat kept at 30° C. The capillary was attached at both ends by well-fitting corks coated with a lacquer insoluble in benzene. The method of calculation has been given elsewhere (1).

In the following condensed tables, P is the pressure in cm. of solution, V the velocity of flow in cc. per 10 sec. and $\eta = \frac{P}{V}$ the relative viscosity. Tables I, II and III express the results for natural rubber sols of various concentrations.

A quantity of acetone-extracted smoked sheet (treatment described above) was placed in large flasks together with a sufficient volume of pure benzene to leave a supernatant layer after swelling of the rubber. The rubber was allowed to diffuse into the liquid for two periods of four days each with withdrawal of supernatant sol and replacement with pure solvent after each period. Care was taken not to shake the vessels during this treatment. The concentration of solute was determined on an aliquot portion and the sol tested for nitrogen by the sodium fusion test. The latter yielded negative results. Approximately 5% of the total weight of rubber diffused into the benzene during this treatment. The relative viscosities of sols of various concentrations at varying pressures are shown in Tables IV, V, VI and VII.

Two of the sols, one of natural and one of the purified rubber, were exposed for six hours to ultra-violet light in a mercury arc fluorescence cabinet. The sols were contained in loosely stoppered quartz flasks with air above the sol. A blue filter was used to avoid rise in temperature. The relative viscosities of these sols after exposure are shown in Tables VIII and IX.

TABLE I
VISCOSITY OF SOL, 0.96%

<i>P</i>	<i>V</i>	η
43.82	0.131	334.0
37.67	0.100	376.7
31.97	0.089	358.6
27.10	0.073	369.6
23.12	0.060	385.4
19.83	0.050	396.6
17.12	0.042	410.9
14.82	0.036	413.5
12.85	0.031	416.6
11.34	0.027	424.9
9.19	0.022	427.2
7.99	0.019	427.9
6.96	0.016	430.5
6.06	0.014	432.8
5.29	0.012	434.8

TABLE II
VISCOSITY OF SOL, 0.38%

<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η
45.5	0.71	64.3	8.33	0.11	75.7
41.5	0.64	65.0	7.69	0.10	74.4
37.85	0.58	65.8	7.10	0.10	73.4
34.60	0.52	66.7	6.53	0.09	72.5
31.63	0.47	66.8	6.03	0.08	75.4
28.92	0.43	67.3	5.58	0.075	74.4
26.48	0.39	68.5	5.14	0.070	73.4
24.28	0.35	69.7	4.75	0.063	75.0
22.30	0.32	70.1	4.38	0.062	71.0
20.46	0.29	70.1	4.01	0.057	70.8
18.80	0.26	71.4	3.75	0.050	74.0
17.30	0.24	71.6	3.41	0.048	70.6
15.91	0.22	72.9	3.13	0.043	72.2
14.69	0.20	72.7	2.89	0.043	66.7
13.50	0.19	72.3	2.62	0.040	65.5
12.45	0.17	74.7	2.41	0.035	68.0
11.50	0.15	75.0	2.20	0.033	66.0
10.61	0.14	74.0	2.01	0.032	63.5
9.78	0.13	73.4	1.83	0.028	64.6
9.01	0.12	74.0			

TABLE III
VISCOSITY OF SOL, 0.19%

<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η
45.60	1.42	32.2	15.15	0.46	32.9	5.09	0.16	31.8
41.50	1.28	32.3	13.82	0.42	32.9	4.62	0.15	30.8
37.90	1.15	32.9	12.63	0.38	32.9	4.20	0.14	30.0
34.60	1.06	32.6	11.53	0.35	33.3	3.81	0.123	30.9
31.55	0.98	32.3	10.56	0.32	33.0	3.48	0.110	31.6
28.75	0.88	32.8	9.62	0.29	32.8	3.15	0.110	28.7
26.30	0.81	32.5	8.80	0.26	33.4	2.83	0.100	28.3
23.90	0.74	32.2	8.04	0.25	32.6	2.56	0.087	29.6
21.85	0.67	32.8	7.33	0.23	32.3	2.31	0.080	28.9
19.90	0.61	32.5	6.68	0.20	33.4	2.09	0.070	29.9
18.17	0.55	32.8	6.13	0.18	33.5	1.90	0.067	28.5
16.58	0.50	32.9	5.58	0.17	32.2			

TABLE IV
VISCOSITY OF SOL, 0.11%

<i>P</i>	<i>V</i>	η
38.85	1.80	21.6
29.22	1.42	20.6
21.72	1.09	19.9
15.97	0.83	19.3
11.69	0.62	18.8
8.47	0.47	18.0
6.06	0.35	17.3
4.26	0.26	16.4
3.21	0.24	13.4
1.70	0.127	13.4
1.06	0.09	11.8
0.49	0.06	8.2

TABLE V
VISCOSITY OF SOL, 0.21%

<i>P</i>	<i>V</i>	η
40.66	1.54	26.4
32.32	1.25	25.8
25.56	1.01	25.4
20.16	0.81	25.0
15.87	0.64	24.9
12.47	0.51	24.6
9.78	0.40	24.4
7.60	0.33	23.4
5.86	0.26	22.5
4.50	0.21	21.4
3.40	0.16	21.3
2.74	0.133	20.6
1.71	0.097	17.6
1.21	0.072	16.8
0.80	0.063	12.7

TABLE VI
VISCOSITY OF SOL, 0.42%

<i>P</i>	<i>V</i>	η
41.22	1.02	40.4
35.31	0.89	39.8
30.56	0.76	40.2
26.32	0.66	40.0
22.68	0.56	40.5
19.58	0.49	40.0
16.89	0.42	40.2
15.24	0.36	42.3
12.57	0.31	40.5
10.84	0.26	41.2
9.36	0.23	40.7
8.01	0.21	39.1
5.46	0.145	37.7
3.95	0.109	36.2
2.81	0.083	32.6
1.95	0.063	31.1
1.28	0.05	25.6

TABLE VII
VISCOSITY OF SOL, 1.06%

<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η
42.7	0.27	156.2	16.64	0.101	165.0	5.57	0.034	162.6
40.39	0.26	156.0	13.39	0.082	163.8	4.44	0.029	154.2
32.21	0.20	160.2	10.79	0.065	165.6	3.52	0.023	153.0
25.79	0.16	161.4	8.68	0.052	168.0	2.77	0.018	158.4
20.67	0.126	163.8	6.96	0.047	148.8	1.93	0.015	133.2

TABLE VIII
VISCOSITY OF PURIFIED RUBBER SOL, 1.06%

<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η
42.8	0.56	77.1	15.82	0.21	77.2	5.61	0.077	73.2
39.65	0.53	75.5	14.64	0.19	77.7	5.18	0.072	72.3
36.70	0.47	78.6	13.57	0.18	77.5	4.76	0.065	73.2
34.05	0.43	78.6	12.55	0.16	76.8	4.40	0.063	69.6
31.50	0.41	77.7	11.61	0.15	76.6	4.00	0.062	64.8
29.20	0.37	79.6	10.74	0.14	76.7	3.66	0.057	64.8
27.10	0.35	78.2	9.93	0.132	75.4	3.33	0.053	62.5
25.05	0.32	77.4	9.17	0.122	75.4	3.03	0.047	63.0
23.23	0.30	78.3	8.48	0.115	73.8	2.78	0.043	64.2
21.50	0.28	78.2	7.80	0.107	73.2	2.52	0.040	63.0
19.93	0.25	79.2	7.20	0.097	74.7	2.30	0.037	62.7
18.48	0.24	78.0	6.65	0.092	72.6	2.08	0.035	59.5
17.10	0.22	77.6	6.10	0.087	70.4			

TABLE IX
 VISCOSITY OF NATURAL RUBBER SOL, 0.96%

<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η	<i>P</i>	<i>V</i>	η
45.55	0.23	197.5	20.40	0.086	238.5	9.80	0.040	248.4
41.70	0.20	207.9	18.93	0.078	241.6	9.13	0.036	252.9
38.33	0.18	214.9	17.59	0.073	241.7	8.49	0.034	250.6
35.28	0.16	218.2	16.31	0.068	240.7	7.90	0.033	241.0
32.52	0.15	221.8	15.16	0.062	245.9	7.32	0.031	239.6
30.00	0.13	225.0	14.09	0.057	246.2	6.81	0.027	250.2
27.73	0.12	229.0	13.10	0.053	248.2	6.35	0.026	248.4
25.64	0.11	230.8	12.19	0.049	249.3	5.90	0.024	241.4
23.73	0.10	234.7	11.35	0.046	249.1			
22.01	0.093	237.2	10.55	0.043	246.6			

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THE SPECIFIC HEAT OF MONEL METAL BETWEEN -183° AND 25° C.¹

BY W. F. HAMPTON² AND J. H. MENNIE³

Abstract

The heat capacity of Monel metal over the range -183° to 25° C. has been measured in an adiabatic calorimeter. By differentiation of the equation representing the heat capacity curve, an equation for the specific heat over this range is obtained and values at various temperatures calculated. The specific heat at 20° C. is found to be 0.0997.

Introduction

In a recent paper (3) a description was given of a Monel metal container used in connection with some calorimetric measurements which are being carried out in this laboratory. During the course of this work it was necessary to determine the heat capacity of the container over the temperature range from -183° C. to +25° C. In the present article a calculation is made from these determinations of the specific heat of Monel metal over this temperature range.

Experimental

In the same paper (3) the form of the container and its particular features were described in detail. It is made entirely of Monel metal except for a thin lead washer placed underneath the cover.

The heat capacity measurements were made in the adiabatic calorimeter designed by Barnes and Maass (1). The temperatures of the thermostat in which the container was brought to a constant temperature before introduction into the calorimeter were obtained by means of a calibrated platinum resistance thermometer. The bath consisted merely of a wide-mouthed Dewar flask. For 0° C. an ice-water mixture was used, and for -78.5° C., solid carbon dioxide moistened with ether. Liquid air was used to give the lowest temperature, -183° C. Points between -78.5° and 0° C. were obtained with acetone and solid carbon dioxide. The bath liquid for the point at -130° C. was gas machine naphtha boiling below 30° C. This was brought to the desired temperature and maintained at this point by means of liquid air blown at regular intervals into a very thin copper tube suspended in the bath liquid. It is estimated that with careful manipulation the thermostat temperature could be controlled to about 0.2° at -130° C. and easily to 0.1° at temperatures above -78.5° C.

Temperature changes in the calorimeter were measured with a calibrated Beckmann thermometer. All heat capacity calculations are made to 25° C.

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Results

The values for the total heat capacity of the container at different initial temperatures are given in Table I. These when plotted gave a smooth curve, and values for the heat capacity of the container used in subsequent calculations were read from this curve. Subtracting from the value for the heat capacity of the container at any temperature that portion due to the lead washer gives the heat capacity for the weight of Monel metal in the container. From this, the heat capacity per gram of the metal is obtained. The weight of the lead washer was 3.0309 gm. and for the purpose of calculation, the value 0.0300 obtained by Richards and Jackson (5) for the average specific heat of lead between -180 and 20° C., was used. The weight of Monel metal was 132.9050 gm.

Values for the heat capacity per gram of Monel metal calculated for different temperatures as outlined above are given in Table I. Upon plotting these values a smooth curve was obtained and from this curve the equation for the heat capacity of Monel metal was found to be:

$$H = -2.503 + 0.09948 t + 0.00001684 t^2 - 0.0000004165 t^3.$$

Differentiation of this equation leads to the following expression for the specific heat of Monel metal over the temperature range from -180° to 25° C.

$$C_p = 0.09948 + 0.00003368 t - 0.000001250 t^2.$$

Values for the specific heat calculated from this equation are given in Table I. They are found to be in good agreement with tangents drawn to the heat capacity curve.

Discussion

Unfortunately no sample of the Monel metal used in the container was available for analysis and its exact composition is not known. A typical analysis of Monel metal, supplied by the International Nickel Company, is as follows: nickel, 68%; copper, 29%; iron, 1.45%; carbon, 0.13%; manganese, 1.04%; silicon, 0.03%; sulphur, 0.008%. Taking the specific heats at 20° C. (4) of nickel as 0.1064, copper 0.0915, iron 0.1078, manganese 0.1211, the value calculated from the above analysis for the specific heat of Monel metal at 20° C. is 0.1017, assuming that the specific heats are strictly additive. This is in reasonably good agreement with the value here found, 0.0997. The average specific heat of Monel metal between 20° C. and 1270° C. has been reported as 0.128 calories per gram (6).

The results for the specific heats at the lower temperatures are submitted with some reserve. The method of carrying out a heat capacity determination involves the transfer of the container from the thermostat into the calorimeter. Ordinarily this process takes less than three seconds and when the temperature is not too low the heat loss during transfer can be neglected. At lower temperatures however, as Barnes and Maass (2) have pointed out, it is very unlikely that the heat loss during transfer is negligible owing to the large difference between the temperature of the container and that of the surrounding air. Moreover, rapid manipulation is more difficult at very low temperatures.

Consequently the values given for the heat capacity at liquid air temperatures are probably somewhat too small.

It may be mentioned that this source of error is of much less importance in the measurement of the heat capacity of materials enclosed within the container. The latter involves the subtraction of the heat capacity of the container at a given temperature from that of the container and contents at the same temperature. Since the heat losses involved in the transfer in each case are small and very nearly equal they tend to cancel.

TABLE I

TOTAL HEAT CAPACITY OF MONEL METAL CONTAINER, HEAT CAPACITY PER GRAM OF MONEL METAL, AND SPECIFIC HEAT OF MONEL METAL

Total heat capacity of Monel metal container		Heat capacity per gram of Monel metal		Specific heat of Monel metal	
Temp., °C.	<i>H</i>	Temp., °C.	<i>H</i>	Temp., °C.	<i>C_p</i>
+25	0	+25	0	+20	0.0997
0	-335.6	0	-2.503	-10	.0990
-26.7	-686.4	-40	-6.412	-40	.0962
-57.8	-1077.9	-80	-10.143	-70	.0910
-78.5	-1339.5	-120	-13.461	-100	.0836
-132.6	-1925.5	-140	-14.899	-130	.0740
-184.2	-2371.7	-160	-16.200	-160	.0621
		-180	-17.434		

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and *E* closed with the sample at atmospheric pressure. The levelling bulb of the burette is raised to a point above *D*.

The spiral *S* is now heated to bright redness and the levelling bulb of the pipette lowered so as to place the oxygen in *A* under a slightly reduced pressure. With *E* still closed, *B* is slowly opened to *CG*. The mercury in *CG* falls into *A* and a small amount of the gas is drawn through the jet, where it burns with a momentary flicker. The remainder of the gas in the burette is now passed in by slowly releasing *E*.

Complete combustion is assured by the fact that the gas to be burned impinges directly on the hot spiral, the combustion being also facilitated by the presence of the platinum jet. Thus there is no danger of the accumulation of unburned gas in the pipette.

At the end of the combustion, the gas is drawn into the burette through *F*, and capillaries *F* and *G* filled with mercury up to *D*.

A pipette of this design has been in continuous use for more than a year in these laboratories in connection with natural gas research. No explosions have occurred in it and it has given complete satisfaction.

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Page 235, Reference 1, for "Arch. Dermatol. Syphilis" read "Arch. Dermat. and Syph".

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